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Autumnal swarming of bats (Chiroptera) in Britain

Katharine Natasha Parsons

A dissertation submitted to the University of Bristol in accordance with the requirements of the degree of PhD in the Faculty of Science

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ABSTRACT

Swarming of bats at underground sites during late summer and early autumn is a topic that has intrigued and confused researchers for several decades. Why hundreds or thousands of bats of several species gather at sites used for hibernation at this time has been subject to some debate. In this thesis I present a series of studies examining aspects of swarming among Britain's resident bat species in a quest to discover the function of swarming and its implications for bat conservation. Swarming activity was monitored at nine underground sites in southern England to assess when swarming occurred, which species took part, the demography of swarming populations and to assess the conservation importance of such sites to Britain's bat community. Swarming was particularly prevalent among *Myotis* species, in particular *M. nattereri* and *M. daubentonii*, but revealed surprisingly large numbers of rare species such as *M. bechsteinii* and *B. barbastellus* also. These surveys confirmed that the characteristics typical of swarming elsewhere (strongly male biased sex ratio and temporal segregation in peak activity between species) were also characteristic in Britain. During observations it was evident that activity during swarming varied markedly from night to night. Therefore activity was logged automatically to provide an index of activity that could be compared with environmental variables. Swarming activity positively co-varied with ambient temperature and was negatively associated with rainfall. Automatic logging was confirmed to be a reliable alternative to catching to provide an index of the number of bats visiting a swarming site that could be used in long-term population monitoring for conservation purposes. Low rates of return of marked bats indicated that populations sizes must be large and catchment areas extensive. Swarming populations were estimated by using mark-recapture techniques to be around 150 *M. bechsteinii*, 1000 *M. daubentonii* and 4000 *M. nattereri* at the main study site. Minimum catchment areas for *M. daubentonii* and *M. nattereri* were estimated as 254 and 497 km^2 respectively and the maximum range traveled from a swarming site for both species was nearly 40 km. *M. daubentonii* were associated with parkland habitats containing deciduous woodland and open water. *M. nattereri* roosts were located in areas of mixed agriculture and they showed a preference toward agricultural land and deciduous woodland for foraging. Few individuals returned to the swarming site, instead they were faithful to compact home ranges of up to 3.4 km^2 and they seldom travelled more than 3 km from their day roost during the night. Peak reproductive condition of swarming male bats was correlated with their visitation of swarming sites. Reproductive condition was further advanced in males with better body condition. Genetic variation among *M. nattereri* at swarming sites was not significantly different from that at maternity colonies indicating that gene flow operates over a wide area, supporting the hypothesis that mating occurs at swarming sites. The protection of swarming sites will conserve bats over large areas and will help maintain genetic diversity.

For Mark

"You are the bearer of unconditional things,

You held your breath and the door for me,

Thanks for your patience."

Alanis Morrisette

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AUTHOR'S DECLARATION

This thesis is the result of my own original work, except where due acknowledgement has been made. Frank Greenaway and Roger Ransome (BatPro Ltd. on behalf of Bath and Northeast Somerset Council) constructed, maintained and ran activity loggers and gave me their data for analysis. Frank Greenaway also supplied me with catch data. Ian Davidson-Watts and Steve Laurence supplied catch data and carried out ringing of bats on my behalf. Freda Marshall undertook microsatellite analysis of DNA samples collected by me, and supplied me with the raw data for analysis. I carried out the majority of catching surveys and all of the radio-telemetry, assessment of bat reproductive status and body condition and biopsy sampling for this study. The literature research, writing, data analyses and conclusions contained in this thesis are entirely the result of my own work.

No part of this work has been submitted in any previous application for a higher degree.
The views expressed in this thesis are my own and not those of the University.

Katharine Natasha Parsons KNP **Date** 04 November 2003

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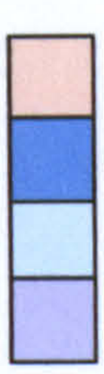
LIST OF ABBREVIATIONS AND COLOUR KEY

Throughout this thesis I use abbreviations and colour codes for species, study sites and habitat types. These are given with all figures and tables but a list is provided here for reference.

Species name	Species abbreviation	Colour code
Barbastella barbastellus	Bb	
Eptesicus serotinus	Es	
Myotis bechsteinii	Mbe	
Myotis brandtii	Mbr	
Myotis daubentonii	Md	
Myotis mystacinus	Mm	
Myotis nattereri	Mn	
Plecotus auritus	Pa	
Pipistrellus nathusii	Pn	N/A
P. pipistrellus/pygmaeus	Pp	
Rhinolophus ferrumequinum	Rf	
Rhinolophus hipposideros	Rh	
Category 'Other' (species specified as necessary)		

Study site	Abbreviation
Box	Box stone mine
Byf	Byfield stone mine
Chi	Chilmark stone mine
Coc	Cocking Tunnel
Dro	Drover's Tunnel
Far	Farleigh stone mine
Fon	Fonthill grottoes
Sav	Savernake Tunnel
Wes	Westhumble chalk mine

Habitat type	Colour code
Amenity	
Arable	
Parkland	
Pasture	
Open Water	
Scrub	
Urban	
Woodland	

CHAPTER ONE

GENERAL INTRODUCTION

1. GENERAL INTRODUCTION

Bats (Chiroptera) are one of the most diverse orders of mammals and the second largest, numbering around 1,001 recognised species (Hutson *et al.*, 2001; Simmons, in press). They are supremely adapted to life in the skies, exploit a wide array of food sources, and follow many different life cycles and mating systems (Nowak, 1994). Bats are classified into two suborders, the Megachiroptera, containing only one family called the Pteropodidae (flying foxes), and the Microchiroptera, of which there are 17 families (Simmons, 1998). Microchiropterans inhabit every continent with the exception of Antarctica. The most widespread and speciose group are the vespertilionids (Neuweiler, 2000), the primary focus of this thesis.

1.1. TEMPERATE-ZONE MICROCHIROPTERA

The extreme climate of the temperate zone has selected for bats with adaptations to survive cold weather and to maximally exploit the insect life that abounds during more favourable times of year. All bat species inhabiting the temperate zone follow a similar annual life cycle, known as the 'temperate cycle' (Bradbury, 1977b; Schober & Grimmberger, 1989) (Fig. 1.1).

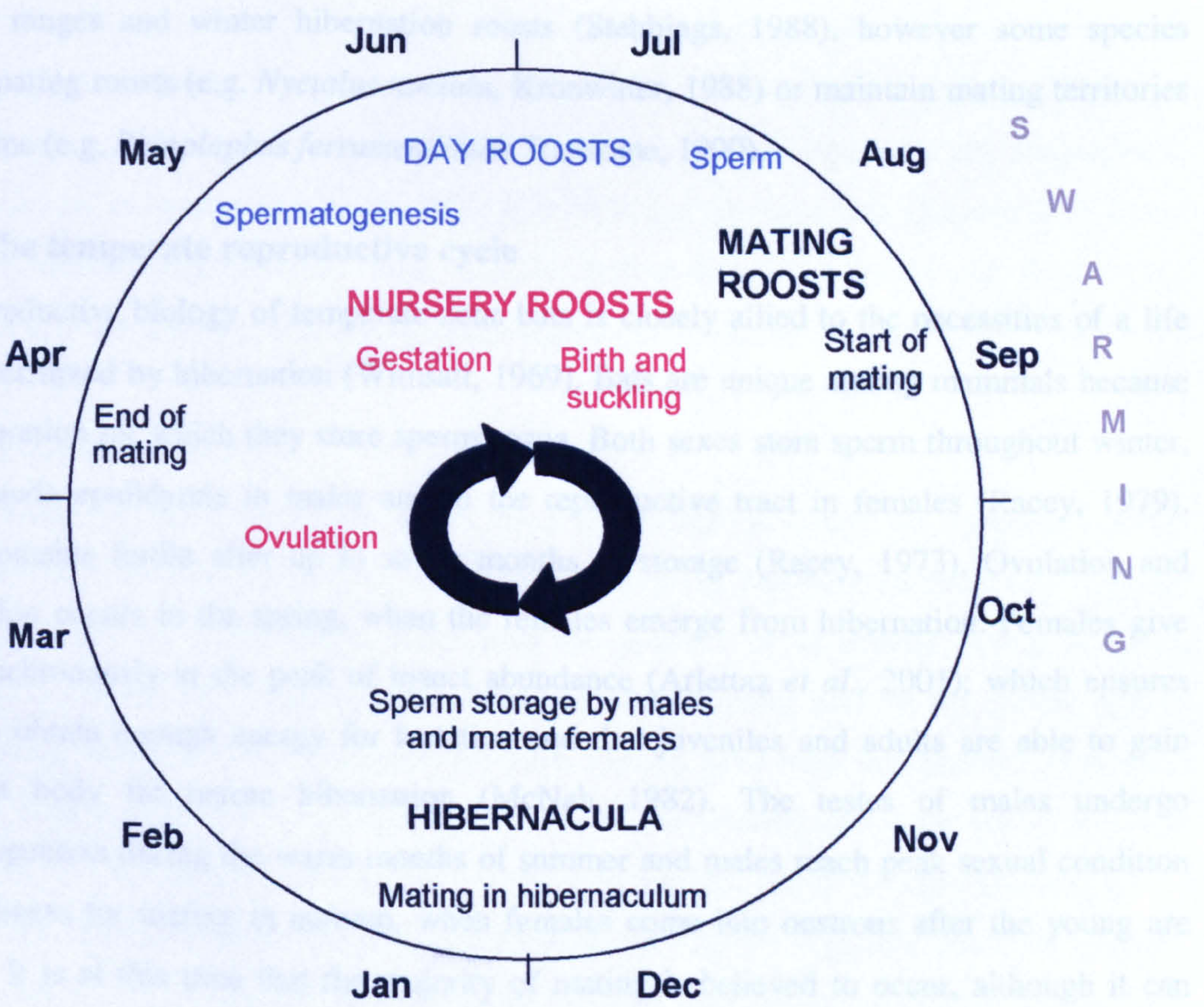


Figure 1.1. The annual cycle of temperate zone bats. Features of males are shown in blue font, females in red and of both sexes in black. Adapted from Schober & Grimmberger, 1989.

1.1.1. The temperate life cycle

During winter, many temperate bat species hibernate, while others migrate to warmer regions. Hibernation is extended and specialized torpor in which the daily arousal process is inhibited (Ransome, 1990). Both families of bats represented in Britain, the Rhinolophidae (horseshoe bats) and the Vespertilionidae (plain-nosed bats) hibernate (Wimsatt, 1969), either underground in caves or mines, or above ground in tree-holes, loft spaces or wall cavities. Hibernation is an adaptation to scarcity of food during winter (McNab, 1982) and generally lasts from November to March, however the exact timing and duration depends on the species and on the latitude at which it lives. In spring females leave hibernacula and gather at traditional nursery roosts where they give birth to and care for their young until mid-summer. Many species exploit wall cavities, roof spaces and crevices in man-made structures, and it is at this time of year that people most often encounter bats. Solitary males or bachelor groups use trees and a variety of structures including buildings as day roosts at this time. Features of hibernation and nursery colonies are well understood for many species because they are accessible to researchers, particularly when in buildings. After weaning, nursery colonies disperse and the whereabouts of most bats is largely unknown prior to hibernation. Many are considered transitory, because they may travel tens or hundreds of kilometres between summer ranges and winter hibernation roosts (Stebbings, 1988), however some species defend mating roosts (e.g. *Nyctalus noctula,* Kronwitter, 1988) or maintain mating territories at this time (e.g. *Rhinolophus ferrumequinum,* Ransome, 1990).

1.1.2. The temperate reproductive cycle

The reproductive biology of temperate-zone bats is closely allied to the necessities of a life cycle interrupted by hibernation (Wimsatt, 1969). Bats are unique among mammals because of the duration for which they store spermatozoa. Both sexes store sperm throughout winter, in the cauda epididymis in males and in the reproductive tract in females (Racey, 1979). Sperm remains fertile after up to seven months of storage (Racey, 1973). Ovulation and fertilization occurs in the spring, when the females emerge from hibernation. Females give birth synchronously at the peak of insect abundance (Arlettaz *et al.*, 2001); which ensures they can obtain enough energy for lactation and that juveniles and adults are able to gain sufficient body fat before hibernation (McNab, 1982). The testes of males undergo spermatogenesis during the warm months of summer and males reach peak sexual condition and readiness for mating in autumn, when females come into oestrous after the young are weaned. It is at this time that the majority of mating is believed to occur, although it can continue throughout hibernation and into the spring. In an action plan for the conservation of bats in the United Kingdom, Hutson (1993) stated that 'relatively little is known of the nature, importance and conservation implication of mating roosts'.

1.2. AUTUMNAL SWARMING

During the late summer and autumn months some bat species exhibit autumnal swarming behaviour (Fig. 1.1). Traditionally the word 'swarming' describes the movement of a large group of animals, for example bees, locusts or rats (Allen, 1984). The term has been applied in two separate instances to bats. The first, 'autumnal swarming' (henceforth referred to simply as swarming) is the subject of this thesis. The second instance is 'dawn swarming' of bats outside the entrances to their summer roosts, which has been described for several species (for example Cross, 1965; O'Shea, 1980; Shiel & Fairley, 2000).

In applying the term 'swarming' to bats Fenton (1969) described aggregations of up to many hundred individuals, often of several species, at cave or mine sites during late summer and early autumn, a behaviour that was first recorded by Poole in North America in 1932 (Fenton, 1969). The first studies of swarming behaviour were carried out in Canada and the USA between the late 1960s and the early 1980s, often as parts of larger investigations of annual activity patterns and social behaviour (e.g. Davis & Hitchcock, 1965; Humphrey & Cope, 1976; Thomas *et al.*, 1979). Several studies in continental Europe during the 1990s found that European bat species partake of similar activities as their North American counterparts (Bauerová & Zima, 1988; Harrje, 1994; Kretzschmar & Heinz, 1995; Kugleschafter, 1995; Lubczyk & Nagel, 1995; Trappmann, 1997). It has only recently been discovered that some British species swarm also (Park, 2000; Parsons *et al.*, 2003 – Appendix 1). Swarming is likely to be an important part of the life cycle of temperate-zone bats (Bauerová & Zima, 1988), probably connected with mating, however, before discussing the possible functions of swarming the characteristic features of swarming will be presented.

1.2.1. Swarming species

Without exception all of the bats that have been documented to swarm are temperate species of the family Vespertilionidae. Reports are solely from the Northern Hemisphere, however this may be due to survey bias, rather than the absence of swarming among Southern Hemisphere species. The behaviour is most prevalent among bats in the genus *Myotis* (mouse-eared bats). In North America, fourteen species have been documented swarming, including nine *Myotis* (Table 1.1). Perhaps one of the best-studied bats in the world is *Myotis lucifugus*, the little brown bat, which is common throughout North America (Harvey *et al.*, 1999). This species swarms in great numbers at the famous Renfrew Mine in Ontario, Canada, and at numerous other locations across the continent. In Europe, eleven species have been documented at swarming sites during the swarming season, including seven *Myotis* species (Table 1.1). It is likely that there are other swarming species that have not yet been documented as such.

Table 1.1. Species cited as swarming in studies from North America and Europe

SPECIES	STUDY
North America	
Myotis evotis	Navo *et al.*, 2000
M. grisescens	Davis, 1964
M. keenii	Davis, 1964; Barbour & Davis, 1969; Fenton, 1969; Hall & Brenner, 1968
M. leibii	Hall & Brenner, 1968
M. lucifugus	Davis, 1964; Davis & Hitchcock, 1965; Barbour & Davis, 1969; Hall & Brenner, 1968; Fenton, 1969; Humphrey & Cope, 1976; Whitaker & Rissler, 1992; Schowalter, 1980; Navo *et al*, 2000
M. septentrionalis	Schowalter, 1980; Whitaker & Rissler, 1992
M. sodalis	Davis, 1964; Hall & Brenner, 1968; Navo *et al*,. 2000
M. subulatus	Davis, 1964; Fenton, 1969
M. volans	Schowalter, 1980; Navo *et al.*, 2000
M. yumanensis	Navo *et al.*, 2000
Eptesicus fuscus	Davis, 1964; Hall & Brenner, 1968; Fenton, 1969; Navo *et al.*, 2000
Lasurius borealils	Davis, 1964; Barbour & Davis, 1969
Nycticeius humeralis	Davis, 1964
Pipistrellus subflavus	Davis, 1964; Hall & Brenner, 1968; Fenton, 1969; Whitaker & Rissler, 1992; Navo *et al.*, 2000
Plecotus townsendii ingens	Clark *et al.*, 1993 and pers.comm.
Europe	
Myotis bechsteinii	Trappmann, 1997
M. brandtii	Lubczyk & Nagel, 1995
M. dasyceme	Trappmann, 1997
M. daubentonii	Degn, 1987a; Bauerová & Zima, 1988; Harrje, 1994; Lubczyk & Nagel, 1995; Trappmann, 1997
M. myotis	Bauerová & Zima, 1988; Lubczyk & Nagel, 1995
M. mystacinus	Lubczyk & Nagel, 1995
M. nattereri	Bauerová & Zima, 1988; Kugelschafter, 1994; Kugelschafter, 1995; Trappmann, 1997
Barbastellus barbastella	Bauerová & Zima, 1988
Eptesicus serotinus	Bauerová & Zima, 1988; Kretzschmar & Heinz, 1995; Lubczyk & Nagel, 1995
Pipistrellus pipistrellus	Kretzschmar & Heinz, 1995
Plecotus auritus	Bauerová & Zima, 1988; Kretzschmar & Heinz, 1995; Lubczyk & Nagel, 1995

Most swarming species are cave dwelling for all or part of their annual cycle (Barbour & Davis, 1969; Macdonald & Tattersall, 2001; Stebbings, 1988). Most hibernate in caves and inhabit a variety of roosts above ground during the summer, most frequently trees or buildings. *P. pipistrellus* is the commonest bat inhabiting buildings in Europe, and is rarely found at underground sites in the UK. Approximately 1000 individuals hibernate at the Heidelberg mine system in Germany where autumnal swarming has been observed (Kretzschmar & Heinz, 1995). The use of underground sites by *P. pipistrellus* is probably the ancestral state and has been largely lost since the provision of castles, churches and other buildings to which it has adapted. Kretzschmar and Heinz (1995) suggested that the autumnal swarming observed at the mine is still performed by building-dwelling *P. pipistrellus* in the form of 'invasions' of houses (Grummt & Haensel, 1966; Smit-Viergutz & Simon, 2000) and may have the same biological function. A species in the same genus, *Pipistrellus subflavus,* roosts in trees during summer but occupies more caves in eastern North America than any other species of bat (Harvey *et al.,* 1999) and it swarms at underground sites (Table 1.1).

1.2.2. Swarming sites

Swarming has been documented at natural caves (e.g. Bauerová & Zima, 1988; Davis, 1964; Humphrey & Cope, 1976; Schowalter, 1980), disused stone and iron mines (e.g. Davis & Hitchcock, 1965; Fenton, 1969; Kretzschmar & Heinz, 1995; Parsons *et al.*, 2003; Whitaker & Rissler, 1992), underground galleries, for example air-raid shelters (Harrje, 1994), in a building covering a well shaft (Trappmann, 1997), the cellar of a castle (Sendor, 2002) and at disused railway tunnels (Parsons *et al.,* 2003). Most of these places are also used as hibernacula during winter by the swarming and other bat species, but not exclusively. For example, Trappmann (1997) found that although *M. daubentonii* and *M. dasycneme* swarmed at a site they did not hibernate there, but other species did. Similarly Zahn & Hager (2002) noted that *M. daubentonii* used small and apparently unimportant hibernation roosts extensively during the swarming season. In general more bats appear to swarm than hibernate at a site (Bilo *et al.,* 1989; Horacek & Zima, 1978 and Leigl, 1987 cited in Kretzschmar & Heinz, 1995). Hall and Brenner (1968) estimated that the winter colony consisted of only 15% of those bats observed swarming. However at many sites bats (particularly vespertilionids) may hibernate out of sight (Roer & Egsbaek, 1966), or may move underground only intermittently during particularly inclement weather, therefore the value of hibernation sites may be easily underestimated.

Most studies have identified site fidelity during swarming, for example Humphrey and Cope (1976) found only one individual at another swarming site during the same season and Davis and Hitchcock (1965) recovered only two out of 73,000 ringed bats at a cave other than

where they were ringed. In most studies, fidelity appeared to exist from year to year as well as during one swarming season. However there is little long-term data on the movements of these bats, which may have revealed use of other sites. Indeed Griffin (1945) found a large proportion of bats in other caves during winters over seven years.

Bats may travel great distances to and from swarming sites. The greatest documented movement is a round trip of over 600 miles (965 km) between a nursery colony and a swarming site completed in less than nine days (Davis, 1964). However, journeys of shorter distances for example, 12.6 km by *Plecotus townsendii ingens* (Clark, 1993 and B. S. Clark pers. comm.) and 14 km by *Plecotus auritus* (Furmankiewicz, 2002) are completed in one night. Avery *et al.* (1984) and Twente (1955) suggested that bats locate the entrances to swarming sites by listening for the ultrasonic calls of conspecifics. This is unlikely because ultrasound attenuates rapidly and hence has very short range (Lawrence & Simmons, 1982). A longer distance form of navigation (perhaps by using spatial maps, geomagnetism or stellar cues) must operate over such great distances.

1.2.3. The swarming season

Autumnal swarming usually commences in late July or early August and continues until September or October (Bauerová & Zima, 1988; Davis & Hitchcock, 1965; Hall and Brenner, 1968). Numbers and species diversity are usually greatest between mid-August and mid-September (Bauerová & Zima, 1988; Davis, 1964; Parsons *et al.*, 2003). Therefore, swarming occurs in the latter half of the Northern Hemisphere summer and the first half of the Northern Hemisphere autumn. The exact timing of onset and termination of swarming depends on the species, latitude and probably also on altitude.

Peak visitation of swarming sites by different species is temporally separated. This is best demonstrated by *M. daubentonii* and *M. nattereri* in Europe (Daan, 1973; Harrje, 1994; Lubcyzk & Nagel, 1995; Parsons *et al.*, 2003; Trappmann, 1997) and by *M. lucifugus* and *M. volans* in Canada (Goad, 1982 cited in Navo *et al.*, 2000; Schowalter, 1980). *M. daubentonii* commence and finish swarming about one month earlier than *M. nattereri,* and similarly *M. lucifugus* swarm earlier than *M. volans.* This observation may be explained by differences in cold-tolerance between the pairs of species meaning that *M. nattereri* and *M. volans* enter hibernation later than the other species and therefore can swarm correspondingly later in the year. Cold-tolerance may be physiological or may be connected to ease of capture of insects by each species due to different foraging strategies (Barclay, 1991) resulting in resource partitioning (Bauerová & Zima, 1989; Kunz, 1973). For example, *M. nattereri* is in part a

gleaning species (Swift & Racey, 2002) and consequently may locate prey later or earlier in the year more easily than an aerial hawking species like *M. daubentonii* (Turner *et al.*, 2002). Some authors have distinguished two phases during autumnal swarming. During the first, flights into the site (in-flights) balance flights out (out-flights) and during the second, in-flights exceed out-flights and bats are found torpid during the day (Fenton, 1969; Kretzschmar & Heinz, 1995). These different phases were not found to be readily distinguishable in the literature or in practice.

A number of authors have also documented a period of swarming during the northern hemisphere's spring (April-June) of lower intensity than that which occurs in the autumn (Bauerová & Zima, 1988; Harrje, 1994; Humphrey & Cope, 1976). This period coincides with emergence from hibernation and the movement of females away from hibernacula toward their maternity roosts. A small peak of activity (at sites used for autumnal swarming) may also occur during the early summer months (Degn *et al.*, 1995; Trappmann, 1997), caused almost exclusively by males that hibernated at the site the previous winter (Harrje, 1994). The sites may be used as transitional roosts because Degn *et al.* (1995) found that more than one thousand bats visited the mine only once during the summer, or perhaps they are familiarising themselves with sites for swarming in the autumn before leaving for summer roosts (Trappmann, 1997).

1.2.4. Nightly swarming activity

Nightly activity during the swarming season commences after dusk, peaks between one and two hours later and continues for several hours before decreasing steadily toward dawn (Bauerová & Zima, 1988; Hall & Brenner, 1968; Harrje, 1994). Humphrey and Cope (1976) stated that activity occurs all night which may be the case during the busiest swarming nights, however they did not comment on whether the intensity of activity changed. Initial activity may be due to the emergence of a few individuals from inside that had spent the day at the site. However, later in the evening animals arrive in large numbers from the surrounding area (Navo *et al.*, 2002).

There is marked variation in activity levels from night to night during the swarming season (Harrje, 1994; Humphrey & Cope, 1976). This is most probably due to the effect of variables such as temperature, rainfall, cloud cover and perhaps insect abundance. Changes in activity levels at two caves in the same region observed by Humphrey and Cope (1976) was synchronous, indicating that swarming activity is affected simultaneously over an entire region and the same population of bats does not simply move to a different cave each night.

Through individual marking of bats with rings it has been found that very few bats are recaptured on consecutive nights suggesting that they leave the area soon after swarming and each night a different group flies in and leaves before morning (Davis & Hitchcock, 1965; Fenton, 1969; Hall & Brenner, 1968; Harrje, 1994; Humphrey & Cope, 1976; Whitaker & Rissler, 1992). Hence the populations visiting such sites are likely to be very large. However, Davis and Hitchcock (1965) found that females departed on the same night but males sometimes stayed during the following day. Conversely, Fenton (1984) suggested that males leave a swarming site after only two hours to avoid becoming torpid in the cool temperature.

1.2.5. Characteristics of swarming communities

Any swarming site is used by a community of bats comprised of populations of more than one species. For the purpose of this study I define a population as a group of individuals of the same species in a particular area at a particular time (Krebs, 1994). A population of bats will be made up of a number of colonies. A colony is defined as a stable group of a single species occupying a definable boundary at a particular time (O'Shea & Bogan, 1999). The bat community at a swarming site is made up of populations of different species from different colonies around the site. I term the area from which bats are drawn to a swarming site as the catchment area of that swarming site. The composition and size of the swarming bat community will depend on the abundance and diversity of bats in the region and therefore will vary from site to site. On average, however, between 4 and 6 species are caught at each site and the numbers of individuals caught per night range from one or two to several hundred (Davis, 1964; Fenton, 1969; Hall & Brenner, 1968; Navo *et al.,* 2002; Schowalter, 1980; Whitaker & Rissler, 1992), although both will also vary according to capture methods and duration of trapping.

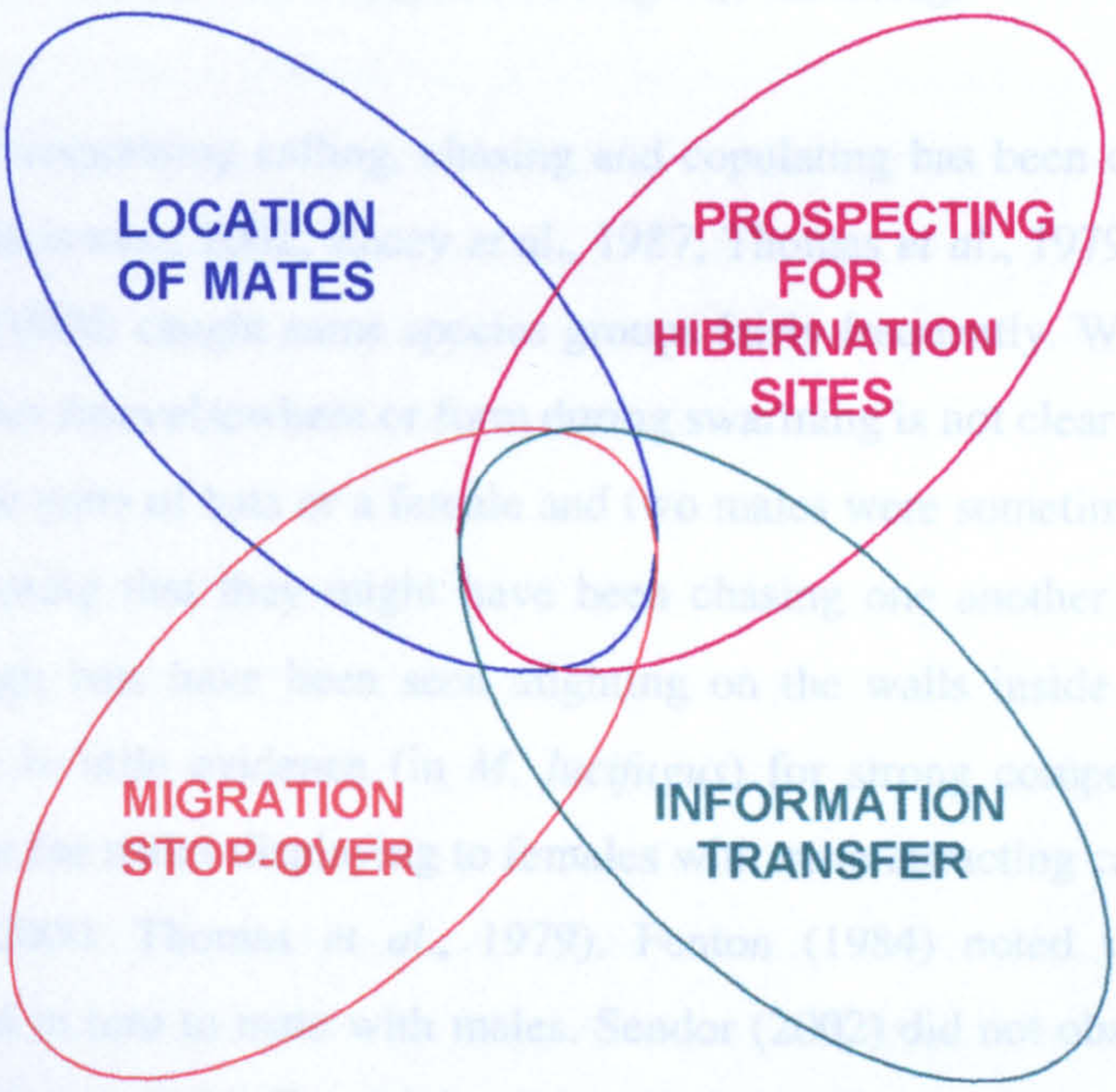
Swarming populations are consistently dominated by males, usually in the order of between 65% and 95% of the total (Bauerová & Zima, 1988; Hall & Brenner, 1968; Hendricks *et al.,* 2000; Navo *et al.*, 2002; Parsons *et al.*, 2003). Sex ratios in spring and winter are usually closer to unity, for example a hibernating population of *Myotis daubentonii* comprised 47% male and 53% female (Harrje, 1994) and hibernating *M. nattereri* comprised 59% male (Stebbings, 1965). Sex ratio of juveniles captured during swarming was near unity in Alberta (Schowalter, 1980). Sex ratio may be highly male-biased at the onset of swarming, lower in the middle and then higher again at the end (Humphrey & Cope, 1976; Schowalter, 1980). Perhaps males gather in advance of the females and juveniles that begin to arrive after the break-up of maternity colonies in the middle of the swarming season. Females might then commence hibernation before males (as found for *M. daubentonii* by Harrje, 1994), causing the sex ratio to become more male biased again toward the end of the swarming season.

Where a greater proportion of juveniles is found late in the season (Harrje, 1994; Schowalter, 1980; Trappman, 1995) this has been explained by juveniles commencing hibernation later than the adults because they require longer to build up fat stores (Schowalter, 1980).

1.2.6. The function of autumnal swarming

The function of swarming has been the subject of much debate since the first papers on the subject in the 1960s. Several theories have been proposed, none of which have been subsequently proved or disproved and which are not mutually exclusive (Fig. 1.2). This makes elucidating the function of swarming particularly difficult. The most favoured explanation of swarming is that it concerns the location of mates, however this could occur at the same time as prospecting for hibernation sites, mothers showing their young where to hibernate or migration between summer and winter areas (Fig. 1.2).

Figure 1.2. Diagram representing the proposed functions of swarming, illustrating that they are not mutually exclusive.



Location of mates:

Swarming could function in locating conspecifics that are otherwise dispersed in the environment during the mating period. Males of swarming species are unlikely to be able to defend individual females because their ranges are not small and exclusive (Komers & Brotherton, 1997) and the costs of attracting and defending a group of females or of

defending the roost used by those females may outweigh the benefits of doing so in an over-dispersed species (Bradbury & Vehrencamp, 1977) (see also section 1.3). Instead therefore, species in which defence of females or of roosts is costly might follow an alternative mating strategy of aggregating at a familiar location, such as a hibernaculum, to meet potential mates.

Fenton (1969) explained the first stage of swarming as a prenuptial "rendez vous" with a high proportion of juveniles from the surrounding area becoming familiar with hibernacula, and the second stage of swarming as the onset of hibernation and the mating period. In a review of mating in vespertilionid bats Fenton (1984) concluded that most mating of *Myotis lucifugus* occurred during August and September in a "disco" mating system at swarming sites. Energetic flight activity, sometimes described as 'frenzied', is seen in the entrances to swarming sites during swarming, indicating a social function. For example, Kretzschmar and Heinz (1995) noted that individual *P. pipistrellus* made between 100 and 1000 flights during one hour of observation in the entrance. Sparks *et al.* (2000) found that the proportion of sexually aroused males (with erect penises) among swarming bats in Indiana, USA decreased over time during swarming, indicating that mating was occurring.

Social behaviour comprising calling, chasing and copulating has been observed (Barclay *et al.*, 1979, Furmankiewicz, 2002; Racey *et* al., 1987; Thomas *et al.*, 1979; Trappmann, 1995) and Schowalter (1980) caught same species groups fairly frequently. Whether groups travel to the sites together from elsewhere or form during swarming is not clear. During mist netting at a swarming site pairs of bats or a female and two males were sometimes captured in quick succession suggesting that they might have been chasing one another (author, pers. obs.). However, although bats have been seen alighting on the walls inside swarming sites and copulating, there is little evidence (in *M. lucifugus*) for strong competition or aggression between males, or for males displaying to females with mate-attracting calls or display flights (Sparks *et al.*, 2000; Thomas *et al.*, 1979). Fenton (1984) noted that female 'curious onlookers' waited in turn to mate with males. Sendor (2002) did not observe any song-flight calls among swarming pipistrelles. Male pipistrelles usually produce song-flight calls during advertisement flights to attract females to mating roosts (Barlow & Jones, 1997; Gerell-Lundberg & Gerell, 1994; Lundberg & Gerell, 1986). This indicates that mating may not be a function of swarming among pipistrelles, which have previously been shown to exhibit resource defence polygyny (Gerell & Lundberg, 1985) so may have no need to mate during swarming, unlike in other species.

Female *M. lucifugus* may wait to mate with some males more than others indicating that female choice might operate during swarming (Fenton, 1984; Racey *et al.*, 1987; Thomas *et al.*, 1979). However, Wai-Ping and Fenton (1988) found that male body size was not a determinant of mating success. Watt & Fenton (1995) concluded through genetic analysis that reproductive success in *M. lucifugus* was skewed toward certain male lineages but whether female choice occurs or the skew results from some form of post-copulatory sperm competition is at present unclear.

Prospecting for hibernation sites:

Swarming cannot solely be concerned with prospecting for hibernation sites, because if it were a sex ratio near unity would be expected as both males and females require hibernation sites (assuming equal sex ratio among the population overall). Even if, as demonstrated for several species (e.g. Holzhaider & Zahn, 2001; Russo, 2002), the sexes segregate at different altitudes or in different hibernacula, female-biased swarming should be found at hibernacula used predominantly by females, but this is not found.

Information transfer:

In the same way, swarming cannot function solely for the purpose of information transfer from mother to young because, if so, a female bias would again be expected as adult females and their young (assuming equal sex ratio at birth as found by Griffin, 1940; Humphrey & Cope, 1976; Milligan & Brigham, 1993 and examples cited therein) would visit the swarming site and there would be no reason for adult males to visit at all.

That said, information transfer (Humphrey & Cope, 1976; Trappman, 1997) (also referred to as maternal guidance by Sendor, 2002) is likely to form part of the function of swarming because it is difficult to imagine that naïve young could find a small cave entrance in such a vast area without prior knowledge. Repeated visits to swarming sites, although disputed slightly by recapture data, could facilitate spatial learning and fixation of knowledge of the location of potential hibernacula in juveniles (Bauerová & Zima, 1988; Davis & Hitchcock, 1965; Fenton, 1969). Kretszchmar and Heinz (1995) suggested that swarming of *P. pipistrellus* at Heidelberg mine was important in maintaining cohesion of the regional bat population through social activity and information transfer. Juvenile and adult female *P. pipistrellus* remained longer at a swarming site than adult males and non-reproducing adult females supporting the information transfer hypothesis in this species (Sendor, 2002).

Migration stop-over:

The fourth theory proposed for the function of swarming is that it is related to migration of large numbers of bats from summer to winter areas (Whitaker & Rissler, 1992) and is supported by low recaptures of marked bats, particularly within the same year. It is possible that gathering at such sites might aid orientation on a long distance migration. However bats have been found back on their summer ranges after swarming (Davis, 1964; B. S. Clarke pers. comm., Parsons & Jones, in press (see Chapter 5 and Appendix 3), and have been found hibernating at swarming sites, so it would appear that they do not use them as stop-overs on their way to other areas and this hypothesis can probably be discounted for most species.

Feeding:

An additional theory that has not received much attention is that bats are feeding during swarming, however none of the food contained in the stomachs of a sample of bats caught during swarming was the same as in a sample of insects from within the cave (Whitaker & Mumford, 1971). In addition, Sendor (2002) recorded no feeding buzzes from *P. pipistrellus* during swarming and insects occurred at exceptionally small numbers at the site. Hence this theory can be discounted.

A combination of theories:

After leaving nursery colonies females may disperse over a large area and therefore be difficult for males to locate. While prospecting for hibernation sites or leading their young to such sites females represent a clumped resource (hotspot) (Bradbury *et al.*, 1986) that may attract males seeking mates. The system is analogous to that in chironomid insects in which the aerial swarm "may be considered to serve as a meeting place for the two sexes, whereby the chance of mating is increased even under relatively low population abundances" (Tokshi & Reinhardt, 1996). Males might be expected to gather at sites that have the highest levels of female traffic; consequently the best hibernacula might also be the best swarming sites. The mixing of many individuals from a large area at a central location would promote out-breeding and therefore maintain genetic diversity in a population (Davis & Hitchcock, 1965). In chironomids, males and females follow different strategies - the males spend their energy in swarming and the females pass through rapidly for a "quick but guaranteed reward" (Tokeshi & Reinhardt, 1996). This might also explain the high male bias seen in swarming bats and would lead to the prediction that males remain at the site longer or return more often than females. Swarming in chironomids may facilitate female choice, because only the fittest males (genetically or physically) may be able to engage in extended or repeated swarming activity (Tokeshi & Reinhardt, 1996). Therefore females are provided with the best quality males, those capable of journeying from their offshore emergence site in a lake, to a resting

site onshore and back to the swarming site on the edge of the lake. This could perhaps operate in bats also, if only the fittest males (perhaps those with the best foraging success and hence good positive energy budget) are able to travel to and from swarming sites and chase or attract numerous females whilst there. Females need not be so rigorous in mate choice and may instead mate promiscuously if the fitness of all males at swarming sites is comparatively high.

As demonstrated above from the current evidence for and against the various proposed functions of swarming, it is unlikely that one theory can explain swarming in all bat species. In particular, it is evident for *P. pipistrellus* that mating is unlikely to occur during swarming and instead guidance of juveniles to hibernacula by their mothers is the probable function (Sendor, 2002). However there are reasons why for some bat species, locating mates and mating might be an important function of swarming in a mating strategy very different from that followed by *P. pipistrellus.* A detailed examination of mating systems among swarming and non-swarming species might reveal whether swarming, particularly in *Myotis* species, can be explained as a mating strategy.

1.3. BAT MATING STRATEGIES

Attempts have been made to fit bats into the traditional classifications of mammalian mating systems. However, so little is known about the mating phase of many species that classification is difficult and many species do not fit neatly into one category (McCracken & Wilkinson, 2000). Variation in mammalian mating systems arises because of differences in distribution of resources, where the resources are either females or the resources that females require (Emlin & Oring, 1977). The concept of 'defensibility' is central to theories of mating system evolution (Bradbury & Vehrencamp, 1977).

Polygamy (where one individual mates with several individuals of the other sex) will evolve when multiple mates or the resources to attract those multiple mates are defendable by individuals (Emlin & Oring, 1977). Polygamy is also more common in species in which one sex (usually the male in mammals) exhibits no parental care (Emlin & Oring, 1977). If females have very short synchronous periods of sexual receptivity, it will be more difficult for individual males to monopolise multiple females (Emlin & Oring, 1977). Similarly if females are very dispersed in the environment, female groups will not be easily defendable. In brief, if the benefits of defending a territory or a group of females outweigh the costs of doing so, male defence and consequently polygyny (one male mates with several females) is expected. Where the costs of maintaining a territory or defending females outweigh the benefits other mating systems are expected.

The majority of mammal species that have been studied are polygynous (Clutton-Brock, 1989) and indeed most bat species studied to date are also polygynous, although there are examples of monogamy (where one male mates with one female) and polyandry (where one female mates with several males) (McCracken & Wilkinson, 2000) but these two systems will not be discussed here. It should be remembered that mating systems can be considered either socially (what sort of pairings are visible to observers), or genetically (what genetic pairings actually occur). Many apparently monogamous animals have, through genetic studies, been found in reality to have other mating systems, for example through extra-pair copulations.

1.3.1. Polygyny

Female-defence polygyny, where a male or males defend a group of females (often termed a harem), has been recorded for several species, mainly in the tropics, for example *Phyllostomus hastatus* (McCracken & Bradbury, 1981). This system may occur when a group of females form stable social bonds and inhabit the same region year round, so a male can defend the females without incurring much cost. In the temperate-zone, females do not inhabit the same region all year so males are less able to defend a group of females.

Instead, many species exhibit seasonal resource-defence polygyny. In this system, males defend and control resources that are essential to females, such as day roosts or foraging areas. For example, single males of *Pipistrellus pipistrellus* and *Nyctalus noctula* establish territories and defend day roosts during the mating season (Gerell & Lundberg, 1985; Sluiter & van Heerdt, 1966). They advertise their presence to passing females by calling or display flights, and mate with multiple females. Although males attract and mate with several females, females may also mate with more than one partner. Up to 71% of offspring of female *Saccopteryx bilineata* may be fathered by males other than the harem male (Heckel *et al.*, 1999) and *Nyctalus noctula* twins can have two different fathers (Mayer, 1995).

1.3.2. Leks

Leks are aggregations of males that females visit solely for copulation (Höglund & Alatalo, 1995), and have been reported for four species of bat (McCracken & Wilkinson, 2000), but none so far in Britain. Leks are defined by: 1) the absence of male parental care; 2) a mating arena significantly smaller than the normal home ranges of males and females; 3) male territories containing no resources required by the females except the males themselves; and 4) the opportunity for females to select mates whilst at the arena (Bradbury, 1977a; Höglund & Alatalo, 1995; McCracken & Wilkinson, 2000). A high degree of inter-male competition is expected and females should be very selective in their choice of partner. A high level of

male-dominant sexual dimorphism, akin to that seen in the hammer-headed fruit bat (*Hypsignathus monstrosus*) (Bradbury, 1977a) might be expected. This is not seen among any of Britain's bat species.

For bats at swarming sites, it would appear that the first two criteria for defining a lek are easily met, however if females visit the site to assess its potential for hibernation then the site itself is obviously a resource required by females, which would violate the third criterion. In addition, as previously mentioned the skewed male reproductive success found by Watt and Fenton (1995) implies that female choice does operate but this may be made null and void if investment in that choice cannot be protected as may be the case in some species which receive unwanted copulations during hibernation (see Section 1.3.4).

1.3.3. Mating swarms

Alternatively, males might aggregate at a hotspot and mob females which are consequently unable to choose between males. Bradbury & Vehrencamp (1977) termed this aggregation a 'mating swarm' and stated that this is what happens during autumnal swarming. This is analogous to the so-called 'disco' mating system of Fenton (1984) mentioned earlier. By far the majority of temperate bat species have in the past been thought to mate randomly and promiscuously (Fenton, 1984; Thomas *et al.*, 1979; Wai-Ping & Fenton, 1988) thus supporting the idea that females do not choose between mates and that they mate more than once. Promiscuous mating (both males and females mating more than once with different partners) can however be highly structured and non-random (e.g. *H. monstrosus*), and there is no convincing evidence that mating in any bat is random (McCracken & Wilkinson, 2000).

1.3.4. Random mating?

Random mating is not a sensible strategy for most mammals. Female bats invest heavily in very few offspring per year; therefore only really need to mate once, and so could be expected to be very selective in their choice of father for their offspring (Watt & Fenton, 1995). Males on the other hand, invest only spermatozoa and should compete with other males to mate with as many females as possible to maximize their mating success. However, because mating can continue throughout hibernation some temperate-zone bats are unable to protect investment in mate choice or competition (Thomas *et al.*, 1979; Wai-Ping & Fenton, 1988).

In some hibernating species, for example *Rhinolophus ferrumequinum* (Oh *et al.*, 1983 cited in McCracken & Wilkinson, 2000), *Rhinolophus hipposideros* (Gaisler, 1966), *Nyctalus noctula* (Grosser, 1903 cited in Fenton, 1984) and *Pipistrellus pipistrellus* (Racey, 1979),

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vaginal (copulatory) plugs form after copulation to prevent sperm leakage and insemination by other males. These plugs have different origins depending on the species. In horseshoe bats, the plug is formed from coagulated secretions of male origin (Racey, 1975). This plug may protect any energetic investment that males have made in competing with one another or in advertising displays to attract females, by increasing their chance of successful fertilization by preventing other male's sperm from entering the female. In *N. noctula* and *P. pipistrellus,* the plug is formed from hypertrophy of connective tissue or cornified epithelial cells in the vagnia (Racey, 1979) and is therefore of female origin. This may protect any investment that females have made in choosing a particular male to father her offspring, by reducing the chance of fertilization by the sperm of subsequent, possibly unwanted males that may mate with her. Such females are protected from insemination by the sperm of unsolicited males while they are torpid.

Females without vaginal plugs might receive unsolicited copulations while torpid, and in addition males are unable to defend females they have mated with (Fenton, 1984). Therefore in these species it may pay both males and females to copulate as often as possible without investing time and energy in competing with one another or in choosing partners. Vaginal plugs have not been found among the *Myotis,* yet despite this inability of protecting investment in mating during hibernation Watt & Fenton (1995) found that some males or male lineages in *M. lucifugus* father more young than others, suggesting the following possibilities: that female choice does operate during mating; that there is preferential survival of sperm of certain males during sperm storage; that some males produce larger ejaculates; or perhaps there is an effect of the order of mating, where either the first or last male to mate obtains paternity (Hosken, 1998). Fenton (1984) suggested that sperm from males that mated with torpid females might obtain a competitive advantage over sperm stored from autumn mating but there is no conclusive evidence of last male advantage (Hosken, 1998). Whatever the mechanism, the result is that copulations among *Myotis,* although promiscuous, may not be random. On the other hand if copulations do occur randomly, some form of sperm competition intervenes and the outcome is not random, but instead is skewed towards certain males.

Swarming may represent a mating system whereby bat species that are unable to defend mating roosts or harems and cannot protect their investment in reproduction, obtain as many copulations as possible. The wide dispersion and low population density of many of these species may necessitate meeting at a familiar location to find mates, a function performed by swarming.

1.4. BATS IN BRITAIN

Britain has sixteen resident species of Microchiroptera from seven genera (Macdonald & Tattersall, 2001) (Table 1.2). Two species (*Rhinolophus ferrumequinum* and *R. hipposideros*) are rhinolophid (horseshoe) and the remainder are vespertilionid (plain-nosed) bats. Greatest species richness is found in the south and southwest of England and Wales (Corbet & Harris, 1991), however status and population estimates are poorly known for many species due to lack of data and difficulty of study (Harris *et al.*, 1995; Macdonald & Tattersall, 2001).

1.4.1. Resident species

The most speciose genus of bat in Britain is the genus *Myotis,* containing five species (Table 1.2). *Myotis nattereri* (Natterer's bat) and *Myotis daubentonii* (Daubenton's bat) are the most abundant and widespread of these. The British population of *M. nattereri* is believed to be of European, or even global importance (Hutson, 1993; Macdonald & Tattersall, 2001). *M. nattereri* frequents open woodland and pasture habitats, especially with associated open water or marshland (Seimers *et al.*, 1999; Smith & Racey, 2002). Summer nurseries of between 20 and 200 individuals are usually in roofs of stone-buildings or timber-framed barns, in tree holes or under bridges (Smith & Racey, 2002). This species feeds by gleaning insects from vegetation and spiders from their webs, but also by aerial hawking close to vegetation and the ground (Arlettaz, 1996; Seimers & Schnitzler, 2000; Sheil *et al.*, 1991; Swift & Racey, 2002).

More is known about *M. nattereri* during the mating season than for the other *Myotis* in Britain. Solitary males have been found in bat boxes during autumn with up to 16 females (Altringham & Bullock, 1988), although mixed-sex aggregations with more than one male have also been found in bat boxes at this time (Park *et al.*, 1998). However, with the exception of *R. ferrumequinum* (Rossiter *et al.*, 2000) and P. auritus (Burland *et al.*, 2001), there are no data on the breeding success of males of any British bat species. For example, it is not known whether there is a skew in reproductive success towards one male or whether females mate once or several times. Therefore, little is known of their mating strategies.

M. daubentonii is strongly associated with riparian habitats and nursery roosts are in tree holes, stone buildings or under bridges. Bats of this species forage predominantly on insects swarming above or emerging from rivers, canals and lakes (Rydell *et al.*, 1994; Swift & Racey, 1983) by aerial hawking and gaffing (Jones & Rayner, 1988; Turner *et al.*, 2002). *Myotis brandtii* (Brandt's bat) and *Myotis mystacinus* (whiskered bat) were not distinguished from one species until 1970 (summarized in Baagøe, 1970) hence distribution records are less accurate than for some other species. Both are associated with woodland and have nursery roosts in buildings (Corbet & Harris, 1991).

Myotis bechsteinii (Bechstein's bat) is the most rare of the *Myotis* in Britain and probably one of Britain's rarest resident mammals (Harris *et al.*, 1995). A population of around 1,500 individuals has been estimated (Harris *et al.*, 1995) and four breeding colonies are currently known in the UK. This species is difficult to find and study because they mainly roost in trees, in both summer and winter (Corbet & Harris, 1991). Genetic studies in Germany have shown *M. bechsteinii* to have strong female natal philopatry and strong male dispersal, presumably to avoid inbreeding (Kerth *et al.*, 2000; Kerth *et al.*, 2002).

The commonest bats in Britain are pipistrelles, separated into the common (*Pipistrellus pipistrellus)* and soprano (*Pipistrellus pygmaeus*) species as recently as the early 1990s (Barratt *et al.*, 1997; Jones & Barratt, 1999; Jones & van Parijs, 1993). Both are predominantly house-dwelling and occasionally form maternity roosts exceeding 1000 individuals (particularly *P. pygmaeus*) (Barlow & Jones, 1999). Evidence of differences in natural history, for example in foraging behaviour, between the two species is accumulating (Barlow, 1997; Davidson-Watts & Jones, in prep.; Vaughan *et al.*, 1997). *Pipistrellus nathusii* (Nathusius' pipistrelle) has recently been discovered breeding in Britain and is now considered resident (Russ *et al.*, 2001). These three species are most often associated with buildings and are rarely found at underground sites in Britain, with the exception of disused railway tunnels.

Plecotus auritus (brown long-eared bat) is the next most abundant species after the pipistrelle and roosts mainly in roof spaces during summer (Entwistle *et al.*, 1997). It forages by listening for moths and by gleaning insects from the surface of vegetation (Swift & Racey, 1983). *Barbastella barbastellus* (barbastelle) is rare throughout its range and nursery colonies have only recently been found in Britain, mostly in trees, and one in a barn (Greenaway, 2001). *Eptesicus serotinus* (serotine) mostly roosts in buildings but has a restricted distribution in Britain, confined to the south. It hawks large insects such as beetles and moths over pasture, grassland and parkland (Catto *et al.*, 1996).

R. ferrumequinum and *R. hipposideros* (the greater and lesser horseshoe bats) are both endangered in Britain, however recently both species have shown signs of population recovery, resulting from intensive conservation efforts and legal protection in recent decades (Walsh *et al.*, 2001). Both species are reliant on caves, mines and cellars for hibernation, and spacious lofts for breeding (Ransome, 1991a & b). *Nyctalus noctula, Nyctalus leisleri* and *Plecotus austriacus* will not be discussed here. For further information on these and other species the reader is referred to Harris *et al.* (1995) and Macdonald & Tattersall (2001) for general reviews.

Table 1.2. Chiroptera species list for Britain, with UK status and estimated population size (after Macdonald & Tattersall, 2001 with population estimates and measured of reliability from Harris *et al., 1995*). Reliability is on a scale of 1 to 5, where 1 is good and 5 is poor. It is also noted whether or not each species was captured during this study.

Species	UK status	Estimated population	Reliability of estimate	Caught during this study
Barbastella barbastellus Barbastelle	Rare [V]	5,000	5	Yes
Eptesicus serotinus Serotine	Uncommon Except southeast	15,000	4	Yes
Myotis bechsteinii Bechstein's bat	Very rare [V]	1,500	4	Yes
Myotis brandtii Brandt's bat	Common In W and N	30,000	5	Yes
Myotis daubentonii Daubenton's bat	Common	150,000	4	Yes
Myotis mystacinus Whiskered bat	Uncommon	40,000	4	Yes
Myotis nattereri Natterer's bat	Fairly common	100,000	4	Yes
Nyctalus leisleri Leisler's bat	Scarce	10,000	4	No
Nyctalus noctula Noctule bat	Uncommon	50,000	3	No
Pipistrellus nathusii Nathusius' pipistrelle	Unknown	Unknown		Yes*
Pipistrellus pipistrellus Common pipistrelle	Common	2 million	3	Yes*
Pipistrellus pygmaeus Soprano pipistrelle	Common	2 million	3	Yes*
Plecotus auritus Brown long-eared bat	Common	200,000	4	Yes
Plecotus austriacus Grey long-eared bat	Very rare	1,000	3	No
Rhinolophus ferrumequinum Greater horseshoe bat	Endangered	4,000-6,600	2	Yes
Rhinolophus hipposideros Lesser horseshoe bat	Endangered [V]	14,000	2	Yes

[V] Listed as 'Vulnerable' by IUCN (Hutson *et al.*, 2001) * Caught at tunnel sites only, not at mines

1.4.2. Species protection

All British bat species are protected by British and European legislation (the Bern Convention on the Conservation of European Wildlife and Natural Habitats, 1979; the Bonn Convention on the Conservation of Migratory Species of Wild Animals, 1979; the European Union Habitats and Species Directive, 1992; the Wildlife and Countryside Act, 1981). Many nursery sites of rare species and mixed-species hibernacula are designated Sites of Special Scientific Interest (SSSIs) and some are also candidate Special Areas of Conservation (cSACs). Walsh *et al.* (2001) state that in the future "essential, core habitat" should be incorporated into SACs in addition to roost sites.

The main threats to bats in Britain are losses of nursery roosts and hibernacula and loss or degradation of foraging habitat. Insect diversity and abundance has been reduced following changes in agricultural practice and increased use of insecticides, thereby reducing the amount of food available for bats. Because of bats' gregarious nature destruction of a nursery roost or a hibernaculum is likely to effect bats over a very wide area (Stebbings & Griffith, 1986). Bats need a complex mosaic of habitats and their requirements change during the course of the year. Much additional research is required toward greater understanding of the ecology and behaviour of British bats, to enable effective and strategic conservation planning for their future. Bats were the subject of a national monitoring scheme between 1996 and 2000 to identify methods of monitoring population trends (Walsh *et al.*, 2001). Many of Britain's bat species are difficult to study because they are scarce and are seldom easily located in the field during summer or winter. Research conducted at swarming sites where bats gather during the autumn might provide important information to supplement that gathered by the monitoring programme.

I aim to provide an overview of autumnal swarming behaviour in Britain to demonstrate the importance of swarming sites for Britain's bat species. I will concentrate on various aspects of swarming, listed below. The findings will be of great importance in understanding swarming, furthering our knowledge of the ecology of swarming bat species and will help in identifying the scale over which conservation plans for swarming species must operate.

THESIS AIMS:

1. To describe swarming in British bat species.
2. To correlate swarming activity with environmental variables.
3. To estimate the size of swarming populations.
4. To discover the distance travelled by bats from swarming sites.
5. To study nightly activity and habitat use during the swarming season.
6. To examine reproductive condition of male bats during swarming.
7. To compare genetic composition of bats at swarming sites with those at nursery colonies.

THESIS ORGANISATION:

In **Chapter Two,** I present results of capture surveys at swarming sites in southern England, including details of species composition, sex and age ratios of the swarming bat community.

In **Chapter Three,** I present a more in-depth investigation of swarming at one site where automatic logging of bat activity was used in addition to capture methods. I investigated whether swarming can be predicted from environmental variables and whether logging can be a reliable alternative to capture surveys for monitoring a site.

In **Chapter Four,** I present mark-recapture data from bats ringed and re-captured at the main study sites, including rate of return and population estimates.

In **Chapter Five,** I present information about the distribution of two species around a swarming site with roost types, habitat use and nightly activity during the swarming season.

In **Chapter Six,** I present data on the reproductive and body condition of male bats during swarming and comparisons of male and female body condition and size.

In **Chapter Seven,** I present a genetic study of *M. nattereri* comparing genetic diversity among swarming populations with that at the colony level.

Chapter Eight is a general discussion, ending with conclusions from the study.

Each data chapter is structured as a paper with Introduction, Methods, Results and Discussion. References for the entire thesis are given at the end. All statistical analyses were carried out in Minitab (version 11 for Windows). A critical value of $\alpha = 0.05$ was used in all tests except where otherwise stated. Where data was not normal and could not be transformed, non-parametric statistical tests were performed. Details of the exact statistical tests used are given in each section.

CHAPTER TWO

COMPOSITION OF BAT COMMUNITIES AT SWARMING SITES IN SOUTHERN ENGLAND

2. COMPOSITION OF BAT COMMUNITIES AT SWARMING SITES IN SOUTHERN ENGLAND[1]

SUMMARY

The occurrence of swarming of bats at underground sites in southern England was investigated. Bats were captured by myself and colleagues at nine mine and tunnel sites over a period of eight years (1995 – 2002). 85% of capture events were during late summer and early autumn and the remainder were during spring and early summer. Capture rates were highest between mid-August and mid-September. Capture rates in spring were approximately one-third of those in autumn.

A total of 6237 bats of twelve species was caught. The minimum number of species at any one site was seven and the maximum was ten. *Myotis* bats predominated in autumn. At some sites these are rarely seen during winter hibernation counts. Relatively high numbers of internationally vulnerable species such as *M. bechsteinii* and *Barbastella barbastellus* were recorded. *Myotis nattereri* and *M. daubentonii* were most common.

Species composition changed seasonally. *M. brandtii* dominated early in the season, followed by *M. daubentonii* and finally *M. nattereri.* This progression in peak activity corresponds with the presumed order of the onset of hibernation in the species. There was a significant male bias in captures of swarming species, but not in *Rhinolophus* spp. or *Pipistrellus* spp. Sex ratio was still male-biased among juveniles in the species with the largest sample sizes. Juveniles comprised between 10 and 50% of the total of each species. The highest proportions of juveniles were recorded for the two *Rhinolophus* species.

Some bats, particularly *M. brandtii* early in the season, had rock dust on their forearms and feet indicating that they were either using the site as a transitory day roost, or that they had landed on surfaces while swarming, perhaps during mating.

This study revealed far more species and individuals than would otherwise have been recorded by hibernation counts or bat detector surveys. The conservation importance of swarming sites is considerable, particularly if the main activity of swarming is the location of mates.

[1]A paper based on some of the data contained in this chapter is published with the title 'Swarming of bats at underground sites in Britain – implications for conservation' (2003) *Biological Conservation* **111**: 63-70. G. Jones, I. Davidson-Watts and F. Greenaway are co-authors. See Appendix 1. The paper includes data up to 2000 only.

2.1. INTRODUCTION

The first step in determining whether autumnal swarming {"the flights of bats through hibernacula in late summer and early fall" (Fenton, 1969)} occurred in Britain was to survey underground sites during late summer and early autumn and to compare activity levels, composition of the species community and sex ratios with those at other times of year.

2.1.1. Previous monitoring of underground sites

Prior to this study many underground sites in Britain have been routinely surveyed for hibernating bats during winter, yet have gone unchecked during spring, summer and autumn. Where summer checks take place for breeding colonies, for example of *R. ferrumequinum*, they are usually done during the day. Consequently the species richness of underground sites may have been under-estimated because some species are rarely, if ever, seen in hibernation (for example *M. bechsteinii*), or rarely use underground sites during the day in summer and hence are assumed to be absent from an area. In fact they may be found swarming in greater numbers than expected. For example, between 1958 and 2000 ten bat species were found during hibernation counts at a natural cave in the Czech Republic. When spring and autumn netting at the entrance was introduced in 1991 the species count increased to fifteen, including *M. bechsteinii* and *B. barbastellus* (Gaisler & Chytil, 2002). Autumnal capture surveys therefore complement hibernation counts in describing the distribution of species, particularly those at low population densities such as *M. bechsteinii* and *B. barbastellus*.

2.1.2. Describing a community

Species richness is the simplest measure of the total number of species present in a community and is obtained by sampling (which may be via capturing or sighting the animals or perhaps from indirect signs, such as scats or fur). The number of species recorded increases with sampling effort until an asymptote is reached (Moreno & Halffter, 2000). Species accumulation curves can be used to assess whether a species inventory is likely to be complete and how much effort should be invested in sampling. To include information about the relative abundances of different species in describing a community, indices of species diversity can be calculated. These numerical expressions allow comparison between datasets. However the relative abundance of different species may be dependent on the timing of sampling during the year and on the ecology of the animals.

2.1.3. Why capture?

Capture is a necessary tool in surveying swarming sites for bats because present technology for analyzing recorded echolocation calls of bats cannot distinguish with enough certainty

between different species of vespertilionid bat, especially *Myotis* which are the main swarming species (Parsons & Jones, 2000; Vaughan *et al.*, 1997). Loggers can monitor activity levels to give an index of the number of bats present (see Chapter 3), but if information on species composition, sex and age structure of the swarming bat community is required capture is at present inevitable. Capture also gives the opportunity of marking individuals, with rings or radio-transmitters (see Chapters 4 and 5 respectively).

The rate of capture of bats can be used to delineate the swarming season in time. I predict that *M. daubentonii* will swarm earlier in the season than *M. nattereri* as found previously (Bilo *et al.*, 1989; Harrje, 1994; Lubczyk & Nagel, 1995; Trappmann, 1997) and that there will be a strong male bias in swarming species (Bauerová & Zima, 1988; Davis & Hitchcock, 1965; Hall & Brenner, 1968; Humphrey & Cope, 1976).

The specific aims of this chapter are:

1. To investigate the incidence of swarming at underground sites in southern England.
2. To determine whether swarming varies between different types of underground sites.
3. To delineate when most activity is concentrated at swarming sites.
4. To describe features including species richness, species diversity and change in the composition of the species community with time.
5. To document sex and age ratios of swarming and other species.

2.2. STUDY SITES

This study focused on investigating autumnal swarming at underground bat sites. The term 'underground' is used to describe "any underground (or mainly underground) structures which fulfill the function of a natural cave in the lives of bats" (Mitchell-Jones *et al.*, 2000). Typically such habitats have moderate annual temperature ranges, high humidity and little or no light penetration. The sites studied here included stone-mines (limestone and chalk), man-made grottos and disused railway tunnels.

Broadly speaking my study area extends from Bristol in the west to Dorking in the east (Fig. 2.1), with a focus on the county of Wiltshire, where some of the most extensive stone-mines in the country are situated.

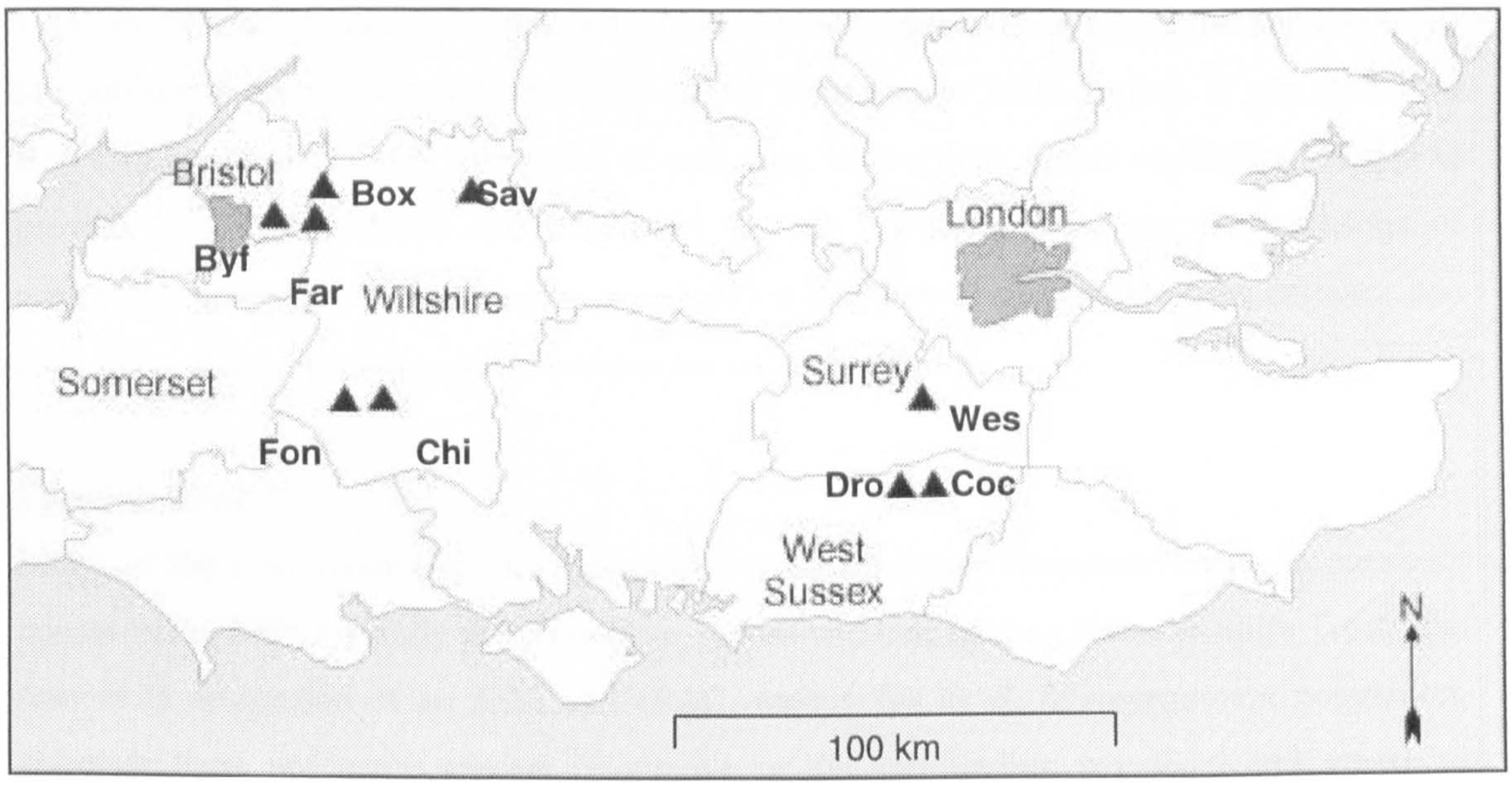
"*Historically the limestone of the Cotswolds and Chilmark area have been mined for stone, and as a result many tunnels and caves were created which are now exploited by bats. Many of the mined areas have also historically retained substantial blocks of woodland which support bats. Rural nature and traditional farming methods in much of Wiltshire retain a mix of arable and pastural land providing a mosaic of habitats essential for many species*" (Anon, Wiltshire Biodiversity Action Plan).

All of the sites are used by at least one bat species during hibernation and two house maternity colonies of *R. ferrumequinum* during the summer months (R. D. Ransome and I. F. Davidson-Watts pers. comm.). The sites were selected because they were already the focus of winter hibernation counts and other bat workers had access for monitoring activities, or because of proximity to other study sites.

2.2.1. Avon and north-west Wiltshire

To the south, east and north-east of the city of Bath there is an abundance of former stone mines known as the Bath Freestone Workings (Price, 1984). Traditionally called 'quarries' despite being underground, these workings are very extensive and limestone from them was exported all over Britain by the canal network and later the Great Western Railway. The beds were mined in long tunnels between 20 and 30 m underground, except at Byfield where the beds are only 1 to 10 m beneath the surface, a contributory factor to massive subsidence in the area in recent years.

Figure 2.1. Map of southern England showing the location of study sites



KEY:

Box	Box stone mine
Byf	Byfield stone mine
Chi	Chilmark stone mine
Coc	Cocking Tunnel
Dro	Drover's Tunnel
Far	Farleigh stone mine
Fon	Fonthill grottoes
Sav	Savernake Tunnel
Wes	Westhumble chalk mine

(Note: Cocking and Drover's Tunnels combined will be referred to as 'Tunnels')

Box mines [ab]

The mine at Box is the largest limestone mine in the UK and possibly the world (Price, 1984). Originally there were ten entrances to the system, which was roughly 2 miles long by 1 mile wide. Five entrances exist today, though some are grilled or locked. Two entrances, Jack's Working (or Candlelight) and Back door were surveyed during this study. Back door was gated to maintain airflow but prevent through-flight of bats and predator access after significant numbers of bat wings were found in early 2002. Hibernation counts are carried out monthly between December and February each year. A breeding colony of *R. ferrumequinum* uses the site during the summer. Box is designated a Site of Special Scientific Interest (SSSI) and a joint candidate Special Area of Conservation (cSAC) with nearby Winsley Mines.

Survey work at each of the sites was conducted by:

[a] Katie Parsons & Gareth Jones, [b] Ian Davidson-Watts, [c] Steve Laurence, [d] Frank Greenaway

Byfield [a]

The limestone mine at Byfield and Firs covered an area of 25 acres when it was closed in 1860. In 1991 all entrances to the Combe Down system were notified as SSSIs. The mine was the subject of an assessment survey by Roger Ransome in 2000 as part of the Bath and Bradford-on-Avon cSAC. The mine is currently undergoing major stabilizing works to prevent further subsidence and to protect the bat habitat. I conducted two seasons of swarming surveys at the main entrance to Byfield, one before and one after the entrance had been altered extensively to provide access for the strengthening works.

Farleigh Mine [a]

Much of the area containing entrances to the Farleigh Down limestone quarry is currently conserved as Brown's Folly nature reserve, owned and managed by Avon Wildlife Trust. The reserve is designated as an SSSI and cSAC, mainly for its *R. ferrumequinum* population, although there are many species of interest in the surrounding woodland and grassland habitats. Two entrances to the mine system were surveyed for swarming activity. Hibernation counts are carried out twice per winter at this site.

2.2.2 North-east Wiltshire

Savernake tunnel [c]

This disused railway tunnel was introduced to the project in 2001 after a ringed *M. nattereri* was found hibernating in the tunnel in winter 2000/2001. Unfortunately due to the positioning of the bat and license restrictions of the surveyors the ring was not read, however it was assumed to have come from Box mines, nearly 40km away. The tunnel has been managed and monitored for bats by Wiltshire bat group for over ten years (Laurence, 2003). They have enhanced the suitability of the site for hibernation of bats by grilling and breeze-blocking the two ends of the tunnel. Over 300 pieces of wood, corrugated Perspex and roofing tiles have been fixed to the tunnel walls and holes have been made to create additional places for hibernating bats. The number of bats seen in hibernation has increased from 40 in 1993 to 129 in 2001 (Macdonald & Tattersall, 2001) to a record 177 in 2002 (S. Laurence pers. comm.). The tunnel is in close proximity to Savernake Forest, one of the most extensive areas of ancient deciduous forest remaining in the country.

Survey work at each of the sites was conducted by:

[a] Katie Parsons & Gareth Jones, [b] Ian Davidson-Watts, [c] Steve Laurence, [d] Frank Greenaway

2.2.3 South Wiltshire

Chilmark Quarries [b]

Of the five RAF reserve depots for ammunition storage during the Second World War, only Chilmark survived the war intact (McCamley, 1998). Catching was conducted at three of the eight entrances and hibernation counts are carried out annually, recording an average of 150 bats per count. In addition to five other species, including both species of horseshoe bat, Chilmark supports the largest wintering population of *M. bechsteinii* in the country (anon, Wiltshire BAP). The quarries are designated as a SSSI and a cSAC.

Fonthill Grottoes [b]

Fonthill Grottoes comprises three underground follies constructed in the late 1700s. Seven species regularly hibernate at the grottoes and the maximum recorded hibernation count is 207 bats, the sixth largest hibernaculum count in Britain. Fonthill is designated an SSSI and is a proposed cSAC due to records of *M. bechsteinii* and *B. barbastellus* from swarming surveys. Catching was conducted at the quarry site where small tunnels are cut into the back of a disused quarry.

2.2.4 Surrey and Sussex

Westhumble chalk mine [d]

Listed as a SAC, Westhumble has been monitored for hibernating bats since 1964, and continually since 1984, and is used by nine of Britain's bat species. The single entrance was initially grilled in the early 1970s and again in 1986 to prevent unauthorized access. In 1996, a building was constructed across the entrances. Naturally absorbed solar energy and air convection allow heat to be retained in the upper levels, encouraging bats to digest food and even breed there. The National Trust owns the site and mine and Surrey Wildlife Trust owns the building.

Cocking and Drovers Tunnels [d]

These disused brick-built railway tunnels, in close proximity to one another, are considered together in the analysis and will be referred to as 'Tunnels'. Hibernation counts are conducted at the tunnels during winter.

Survey work at each of the sites was conducted by:

[a] Katie Parsons & Gareth Jones, [b] Ian Davidson-Watts, [c] Steve Laurence, [d] Frank Greenaway

Note: Capture at Box was initiated by Gareth Jones and Ian Davidson-Watts as a pilot project. Data collected prior to 1999 were collected by them without the assistance of the author. All later surveys at Box and all surveys at Byfield and Farleigh were carried out by the author.

2.3. METHODS

2.3.1. Capture of bats

Bats were caught by myself or by my colleagues (for details see section 2.2) at the swarming sites (Fig. 2.1) between March and November (Appendix 4) in the years 1995 to 2002 inclusive. Not all sites were caught at in each year (Appendix 4). Catching was most intensive during the 'swarming season' (beginning of August to the end of October). At this time catching was mostly limited to once per fortnight to minimize disturbance and to maintain an even distribution of catching throughout the swarming season. Less frequent catches using the same methodology were carried out in spring and early summer to provide comparison with the swarming data. Catch dates were influenced by prevailing weather conditions, for example if a very wet night was forecast, catching was postponed to a following fair day.

Bats were caught using one or two harp-traps (2.4 x 1.85 m, Austbat, Australia or custom-made) and/or one or two mist-nets (6 or 12 m, British Trust for Ornithology, Norfolk, UK) (depending on the location and resources available) placed within or across the entrances to the mines and tunnels at night (Plate 2.1) (Appendix 2). Traps and nets were situated to maximize captures of bats and placement remained as consistent as possible at each site. Most catching began at around sunset and continued for a number of hours depending on the prevailing weather, level of activity and the bat worker. At Box trapping sometimes continued until dawn. Thus sampling effort varied among the different sites in length of trapping and type and number of traps used (Appendix 2).

Measures of bats caught per hour of catching and bats caught per trap per hour were calculated (Appendix 2) to partly account for differences in effort between the sites. However, differences in the placement of traps between sites and thus potential differences in trap effectiveness could not be accounted for. For example, at one site a harp trap may have completely covered a tunnel but at another there may have been a gap through which the bats could pass. Likewise at some sites, traps were located several meters inside the entrance, such as at Box, but at others the net could only be placed outside, for example at Savernake (Plate 2.1). In addition, differences between the different types of capture equipment in catching efficiency could not be accounted for.

2.3.2. Processing of bats

Captured bats were removed from traps and nets as soon as possible after capture and placed in cloth bat-bags before processing. Time of capture, species and sex were recorded for each individual. Most British species are easily identifiable in the hand but cryptic species exist, for example *M. brandtii* and *M. mystacinus*. Males of these species can be distinguished by penis shape; however identifying females to species is far more difficult. Examination of the teeth using a hand-lens to determine the relative sizes of protocones on the pre-molars (according to Yalden, 1993) was the approach favoured (Plate 2.2).

Bats were aged as adults or juveniles (young of the year) according to the degree of ossification of the epiphyseal joints in the finger bones (Anthony, 1988; Racey, 1974a). Separation of juvenile from adult individuals was problematic during the autumn and became more difficult as the season progressed because the epiphyses became increasingly fused with the diaphyses (Thomas *et al.*, 1979). Presence or absence of a darkly pigmented chin spot was noted for *M. daubentonii* (Richardson, 1994) and was looked for in other species. Some recaptured *M. daubentonii* known to be greater than one year of age still had chin spots (Jones & Kokurewicz, 1994; Richardson, 1994), hence presence of a chin spot was not taken as a guarantee that an individual was a juvenile, rather the absence of a chin spot was taken as an indication that the animal was adult.

Sexual maturity was investigated by observing nipples on females and testis size and epididymis pigmentation on males (Racey, 1982; Racey, 1988; Thomas *et al.*, 1979). Bats were weighed inside a bag with a 50g scale (Dr. Scale) to 0.1g. Forearm measurements were taken using plastic calipers to 0.1mm (Plate 2.2). Gareth Jones or I (and on one occasion Joanna Furmankiewicz) took measurements. A paired *t*-test concluded that there was no significant difference between the measurements taken by both of us on the same bats ($t_{0.05, 25} = 0.33$, $P = 0.745$); hence I consider our measurements to be comparable. See Chapter 6 for further discussion of sexual maturity and body mass/forearm relationships of swarming bats.

2.3.3. Data analysis

Although some bats were ringed (see Chapter 4), the results presented here are for the total number of bats caught on each occasion rather than total number of individuals. Very few bats caught and ringed on any evening were caught again later in the same evening, presumably because they were aware of the trap's location.

Species accumulation curves (Begon *et al.*, 1996; Moreno & Halffter, 2000) were constructed to discover how many catches should be performed to reach an asymptote of species richness. Simpson's diversity indices (Begon *et al.*, 1996) were calculated as the reciprocal of the sum of squared proportions of each species relative to the total (P_i) for each site using the following equation:

$$D = \frac{1}{\sum P_i^{\,2}}$$

where D = Diversity index and

P_i = proportion of each species

G-tests were used to test whether observed sex ratios for each species at each site differed from unity, where sample sizes of males and females combined were greater than 10 (Zar, 1974). Chi-square tests (with Yate's correction) were used to test for differences between spring and autumn sex ratios where there were more than 5 individuals per category.

Plate 2.1.
Methods of catching bats

Left - putting a harp trap in position at Box stone-mine

Bottom - mist-netting at Savernake Tunnel

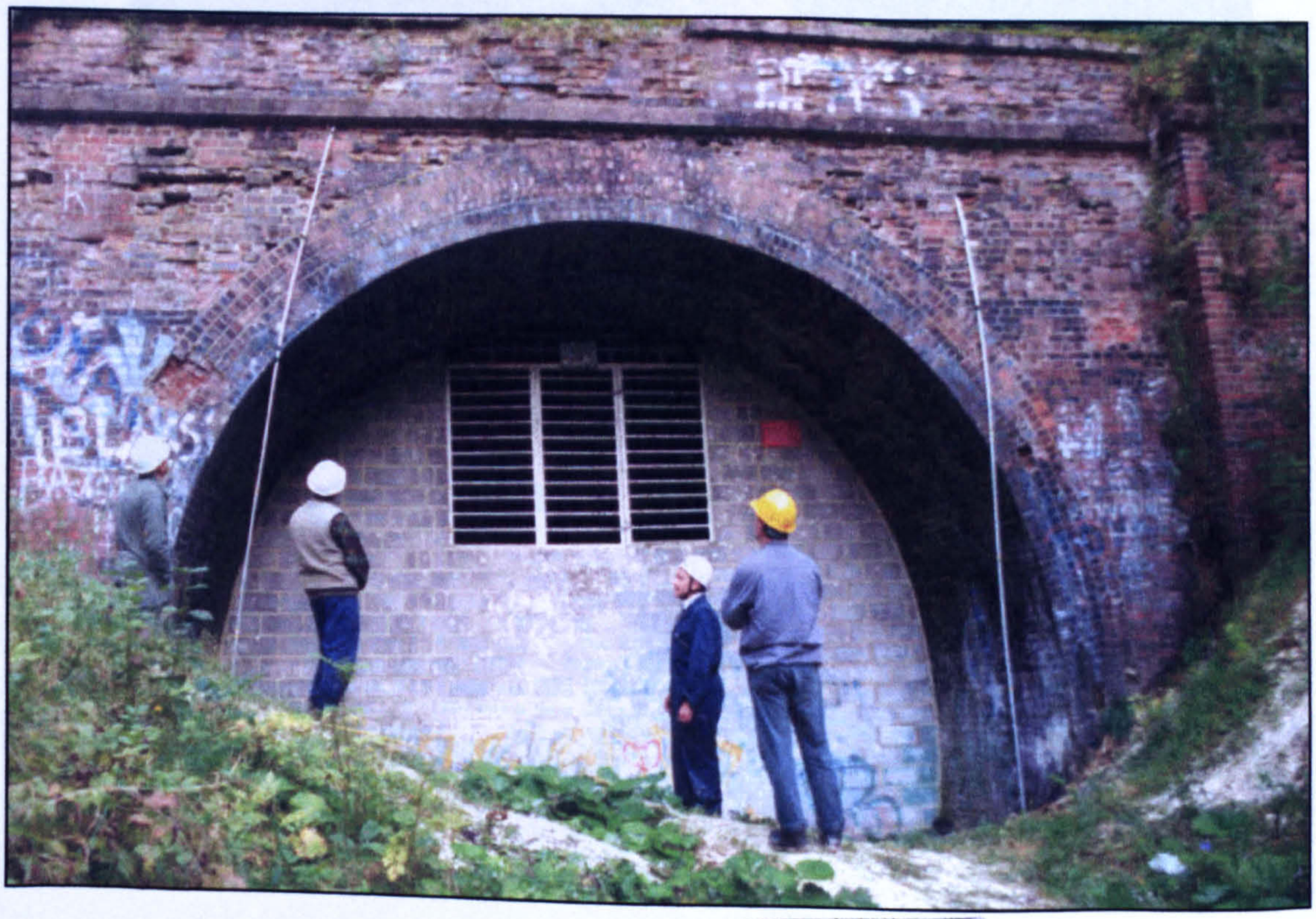


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Plate 2.2.
Processing bats

Left – measuring the forearm of a bat

Bottom – separating *M. mystacinus* from *M. brandtii* by checking teeth

2.4. RESULTS

A total of 6237 bats of 12 species were captured on 177 capture occasions at the nine study sites (Table 2.1). 95.4% of bats were captured during the swarming season (149 catching events) and 4.6% in spring (28 catching events). See Appendix 4 for further information on each catching event.

2.4.1. Capture rate

The greatest number of bats captured at any one site was at Box where 215 bats were captured on 19 September 2002. The highest rate of capture was 16 bats/trap/hr also at Box, on 6 September 1999. At all sites the rate of capture peaked between Julian days 240 and 260 (28 August to 17 September) (Fig. 2.2). The entire swarming period is roughly from the beginning of August to the end of October. There was considerable variation in rate of capture during this time (Fig. 2.2) indicating that the number of bats that swarm (and hence are available for capture) varies markedly from night to night during the swarming period.

Where bats were also caught in spring, the rate of capture was approximately one-third of that during autumn. At Savernake where more spring catching events were undertaken than at other sites, capture rate peaked around day 80 (21 March) after which it decreased steadily to the end of April (Fig. 2.2). Few bats were present at the underground sites between May and July. Three catching events at Box during this time resulted in only five bats in total. At Byfield two catching events in May consisted of only *R. ferrumequinum* and *R. hipposideros* and no other species. The change in rate of captures of bats during the year follows a similar pattern to bat activity recorded by an automatic logging system at Westhumble (see Chapter 3).

2.4.2. Species accumulation

Species accumulation curves for each site show that most have reached an asymptote and no further species are likely to be recorded during capture surveys (Fig. 2.3). The minimum number of species recorded was seven at Westhumble and the maximum was ten at both Byfield and Chilmark. At most sites an asymptote appeared to have been reached after five or six nights of catching, however at Box two additional species were recorded after 23 catching events and at Savernake one additional species was recorded after 33 catching events.

At three sites the number of species captured increased as capture rate increased (Fig. 2.4) and the relationship was significant (Box: $r^2 = 0.51$, $F_{1, 43} = 44.75$, $P<0.0001$; Savernake: $r^2 = 0.26$, $F_{1, 27} = 9.47$, $P = 0.005$; Tunnels: $r^2 = 0.776$, $F_{1, 10} = 34.72$, $P<0.0001$). There was no

significant relationship between number of species and catch rate at the other sites, perhaps due to smaller sample sizes.

2.4.3. Species composition during swarming

Catches during the swarming season were dominated by *Myotis* species at all sites (73 to 97% *Myotis*) (Table 2.1). *M. daubentonii* and *M. nattereri* were most prevalent in both spring and swarming catches, comprising approximately two-thirds of the total (Fig. 2.5). *M. mystacinus, M. brandtii, P. auritus* and *M. bechsteinii* contributed a large proportion of the remainder and were caught with equal frequency during swarming (Fig. 2.5). *R. hipposideros, P. pipistrellus sensu lato, B. barbastellus* and *R. ferrumequinum* were caught in greater proportions during spring than during swarming (Fig. 2.5).

Hotspots for *M. bechsteinii* were identified at Fonthill and Chilmark in south Wiltshire where this species comprised 13.4% and 16.5% of the total catch respectively (Table 2.1). 8.4 and 6.6 individual *M. bechsteinii* were caught per catch at Fonthill and at Chilmark, compared to 2 or less at all other sites.

Diversity indices (D) calculated from catches at each study site permitted a ranking from most to least diverse (Table 2.1) as follows:

Byfield>Farleigh>Chilmark>Tunnels>Box>Westhumble>Fonthill>Savernake

The level of species diversity did not vary with the number of catches at a site ($r^2 = 0.071$, $F_{1,6} = 0.46$, $P = 0.524$). Sites with the most catches were not the most diverse (e.g. Savernake) and sites with relatively few catches (e.g. Byfield) could have a greater diversity score.

2.4.4. Species composition during hibernation

Community composition was very different during hibernation than in spring or autumn, especially in southwest Britain where *R. ferrumequinum* and *R. hipposideros* are most abundant. For example, in two winter counts at Byfield (conducted by R.D. Ransome) 28 and 92 rhinolophids were recorded, but no vespertilionids were seen. Similarly, at Box average annual hibernation counts from 1997 to 2000 (conducted by I.F. Davidson-Watts) comprised 18 *M. nattereri,* 30 *M. brandtii/M. mystacinus,* 11 *M. daubentonii,* fewer than five *P. auritus,* but several hundred *R. ferrumequinum* and *R. hipposideros.* Hibernation counts at the tunnel sites are more similar to the composition seen during swarming. For example at Cocking Tunnel (data supplied by A.M. Hutson, Sussex Bat Group) average hibernation counts

number around 205 individuals, comprising 50% *M. nattereri*, 25–35% *M. daubentonii* and the remainder was *M. brandtii* and *M. mystacinus*.

2.4.5. Change in species composition with time during swarming

The composition of bats captured changed with time during the swarming season. Species richness was greater at the beginning and in the middle of the swarming season than at the end (Figs. 2.6 and 2.7). A clear progression can be seen from dominance of *M. daubentonii* to dominance of *M. nattereri* (Figs. 2.6 and 2.7). At Byfield the pattern may be less clear because *R. hipposideros* comprised a large proportion of the total during October and November, unlike at other sites (Fig. 2.6).

At Byfield and Farleigh (Fig. 2.6) and at Box (Fig. 2.7) a peak in *M. brandtii* occurred early in the season before that of *M. daubentonii.* At Chilmark and Savernake, where catching was not carried out at the beginning of the season, progression in peak activity from *M. daubentonii* to *M. nattereri* was still observed, but I cannot know whether *M. brandtii* would also have been caught there earlier. Only at the tunnel sites were proportions of *P. auritus* and *B. barbastellus* great enough to warrant inclusion in Figure 2.6 in their own categories. *B. barbastellus* declined in frequency with time during the swarming season, whereas *P. auritus* was caught with similar frequency throughout.

The trend for greater species richness early in the season, and progression from *M. brandtii* to *M. daubentonii* to *M. nattereri* were consistent across years, for example at Box (Fig. 2.7). The progression from species to species did not always occur at the same time. In 1999 *M. daubentonii* still comprised around 50% of the total in early October, which was later in the season than in other years. In 2002, *M. brandtii* remained dominant into mid-August (Fig. 2.8), also later than in previous years.

2.4.6. Sex composition of the swarming community

Sex ratio was consistently highly male-biased across all sites for *Myotis* spp. and *P. auritus* (Table 2.2). In 35 out of 36 tests, the observed sex ratio was significantly different from unity (Table 2.2) and in all cases a male bias was evident (59-96% male). The one result that was not significant for *P. auritus* had a sample size of only 12. Sex ratios for *P. pipistrellus, R. ferrumequinum* and *R. hipposideros* were not significantly different from unity. *B. barbastellus* sex ratio was not significantly different from unity at Savernake or Chilmark, but was significantly different at Cocking Tunnel. None of the sex ratios was female biased.

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2.4.6. Sex composition of the swarming community

Sex ratio was consistently highly male-biased across all sites for *Myotis* spp. and *P. auritus* (Table 2.2). In 35 out of 36 tests, the observed sex ratio was significantly different from unity (Table 2.2) and in all cases a male bias was evident (59-96% male). The one result that was not significant for *P. auritus* had a sample size of only 12. Sex ratios for *P. pipistrellus, R. ferrumequinum* and *R. hipposideros* were not significantly different from unity. *B. barbastellus* sex ratio was not significantly different from unity at Savernake or Chilmark, but was significantly different at Cocking Tunnel. None of the sex ratios was female biased.

Limitations in sample size prevented investigating change in sex ratio over time during the swarming season at all sites for all species. At Box, where the greatest number of *M. daubentonii* and *M. nattereri* were captured, average proportions of males (n. males/total) were calculated per fortnightly period from all of the capture data. Fortnightly periods were chosen to break the swarming season into a sufficient number of even length periods for analysis. Sex ratio in *M. daubentonii* became progressively less male-biased during the swarming season (Fig. 2.8a). There was a significant difference in sex ratio between fortnightly periods (ANOVA $F = 3.85$, d.f. = 5, $P = 0.01$, Tukey's pairwise comparisons showed that the sex ratio between 1 and 14 August was significantly different from that between 10 and 23 October). Conversely, sex ratio in *M. nattereri* became increasingly male-biased during the swarming season with a slight decrease at the end (Fig. 2.8b). The difference in sex ratio was significant (ANOVA $F = 2.60$, d.f. = 6, $P = 0.04$, sex ratio between 10 and 23 October was significantly greater than between 29 August and 11 September).

Sex ratio during spring was similar to that during autumn. At Box, for example, 76% of 38 *M. daubentonii* caught during spring were male which was not significantly different from during autumn ($\chi^2 = 621$, d.f. = 1, $P < 0.0001$). Likewise for *M. brandtii* (58% ♂, n=12), *M. nattereri* (77% ♂, n=43) and *R. hipposideros* (50% ♂, n=10) there was no significant difference between spring and autumn sex ratios ($\chi^2 = 202$, d.f. = 1, $P < 0.0001$; $\chi^2 = 1107$, d.f. = 1, $P < 0.0001$ and $\chi^2 = 23$, d.f. = 1, $P < 0.0001$ respectively). Sample size was too small for *P. auritus* and *M. mystacinus* to test statistically but the sex ratios in spring (83% ♂, n=6 & 78% ♂, n=9 respectively) were very close to those seen in autumn (Table 2.2).

2.4.7. Age composition of the swarming community

Chin spots were only clearly obvious in *M. daubentonii* and not in other species. Ageing all species by looking at the shape of their wing joints and the fusion of the epiphyses and diaphyses was particularly difficult as the swarming season progressed. It seemed easiest in *M. mystacinus,* which appeared to have more pronounced tapering of the phalangeal joints (between the fingers bones of the wing) later in the season than other species. Despite my lack of confidence in ageing, *M. daubentonii* and *M. nattereri* classed as adults had significantly greater body condition than those classed as juveniles (Chapter 6), indicating that the majority of classifications were accurate.

Among bats captured at Box (where greatest confidence was had in ageing) between 1999 and 2002 65% of captured bats were aged as adults and 21% as juveniles. For 14% the age was uncertain and was therefore not assigned to either category. Most juveniles were

identified in both *Rhinolophus* species and in *M. mystacinus* (Fig. 2.9). Juveniles are easier to identify in horseshoe bats because there are differences in pelage and the tapering of the joint remains more pronounced for longer. The observation that *M. mystacinus* might have been easier to age later in the year than other species might have meant that the age composition was accurately recorded for this species but not for others. Consequently the proportion of juveniles of other species is likely to have been underestimated. In four species sufficient juveniles were captured to test whether juvenile sex ratio (as seen in Fig. 2.9) differed from unity. In *M. daubentonii, M. mystacinus* and *M. nattereri* sex ratio was biased toward juvenile males at $P < 0.001$; but in *brandtii* sex ratio was not significantly different from unity (all G-tests as per Table 2.2). In the remaining species too few juveniles were captured to statistically test for deviation from unity, although proportions, particularly in *E. serotinus, R. ferrumequinum* and *R. hipposideros,* appear equal (Fig. 2.9).

Monthly age compositions between August and November are presented graphically for male and female *M. daubentonii* and *M. nattereri* (Fig. 2.10). In *M. daubentonii* males and females the proportion of juveniles increases slightly toward the end of the swarming season. In *M. nattereri* the proportion of juvenile males increased from August to September and then decreased to none in November. Juveniles may have been indistinguishable from adults at this time and so some juveniles may have been classified incorrectly. Similarly no females were classified as juveniles during November, perhaps for the same reason. In females of both species the proportion for which age was uncertain increased with time during swarming, but the opposite was seen for males.

Juveniles could not be accurately distinguished from adults in spring on emergence from hibernation so no information is presented for age composition during spring.

Table 2.1. Total number of bats of each species[a] caught during catches at different swarming sites[b] between 1995 and 2002, and Simpson's diversity index (D) for each site.

Site	Number of catches	Bb	Es	Mbe	Mbr	Md	Mm	Mn	Pa	Pn	Pp	Rf	Rh	Total caught	D
Box	52	0	42	106	243	737	283	1251	98	0	0	33	53	2846	3.56
Byf	14	0	2	8	92	67	120	87	12	0	2	63	128	581	6.08
Chi	14	12	4	93	8	169	23	196	47	0	0	5	5	562	4.02
Coc	15	25	0	2	3	149	11	62	59	1	31	0	0	343	3.66
Dro	2	2	0	0	0	23	1	16	6	0	0	0	0	48	
Far	8	0	2	6	17	75	46	98	6	0	0	15	11	276	4.22
Fon	7	1	0	59	3	93	3	271	6	0	0	1	3	440	2.26
Sav	39	21	3	2	1	54	5	360	95	0	19	0	0	560	2.20
Wes	26	0	0	30	5	198	9	319	19	0	0	1	0	581	2.37
Total	177	61	53	306	372	1565	501	2660	348	1	52	118	200	6237*	

[a]Species abbreviations:

Bb = *Barbastella barbastellus,* Es = *Eptesicus serotinus,* Mbe = *Myotis bechsteinii*, Mbr = *M. brandtii*, Md = *M. daubentonii*, Mm = *M. mystacinus*
Mn = *M. nattereri,* Pa = *Plecotus auritus*, Pn = *Pipistrellus nathusii*, Pp = *P. pipistrellus & P. pygmaeus*, Rf = *Rhinolophus ferrumequinum*, Rh = *R. hipposideros.*

[b]Site abbreviations:

Box = Box stone mine, Byf = Byfield stone mine, Chi = Chilmark stone mine, Coc = Cocking Tunnel, Dro = Drover's Tunnel, Far = Farleigh stone mine, Fon = Fonthill grottoes, Sav = Savernake Tunnel, Wes = Westhumble chalk mine

* 7 animals were caught but escaped before idenfication hence they are not included.

Figure 2.2. Rate of capture of bats (number of bats per trap per hour) per catching occasion at the study sites. (Note: no distinction is made between harp traps and mist nets in capture efficiency.)

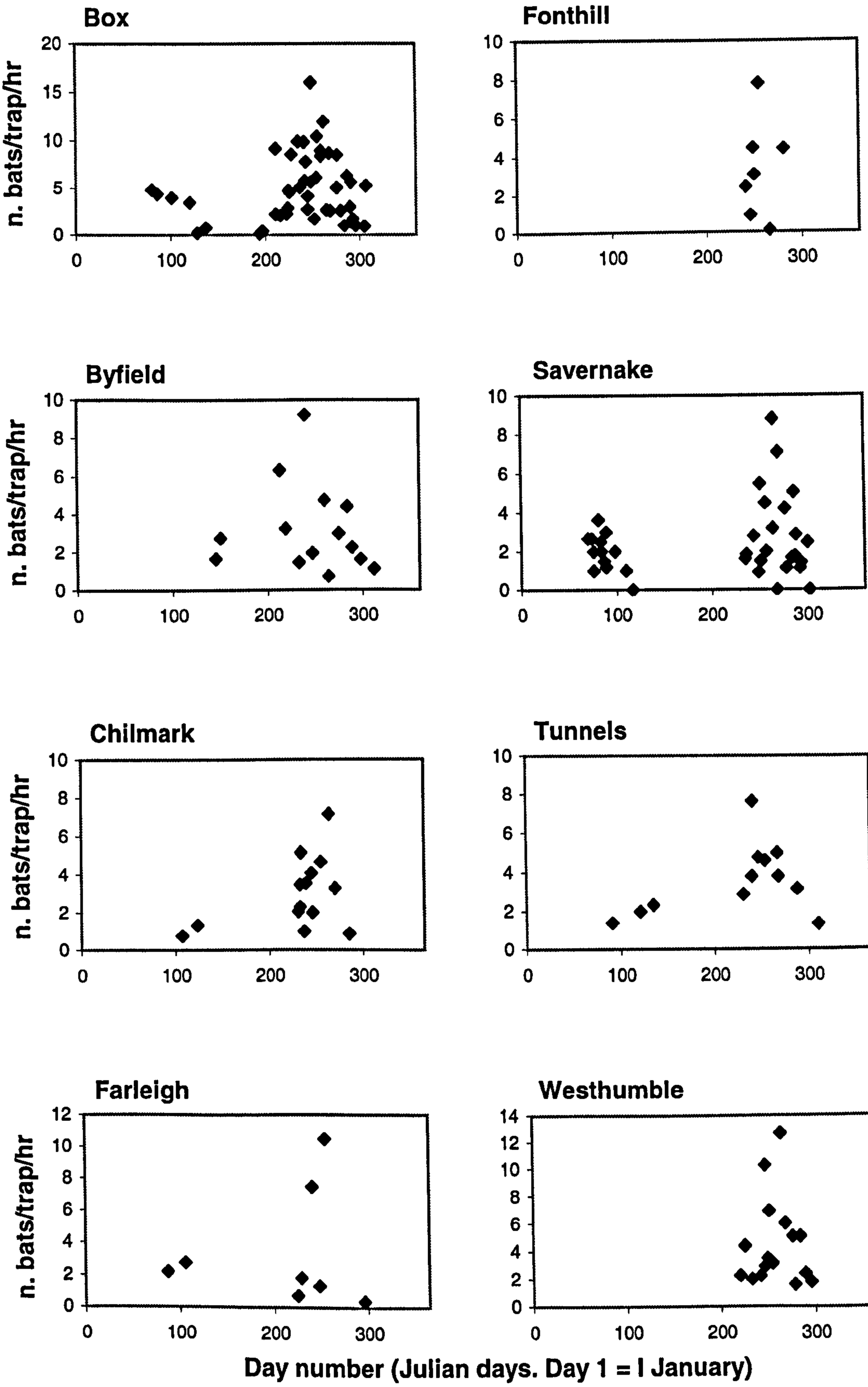


Figure 2.3. Species accumulation curves for each of the study sites

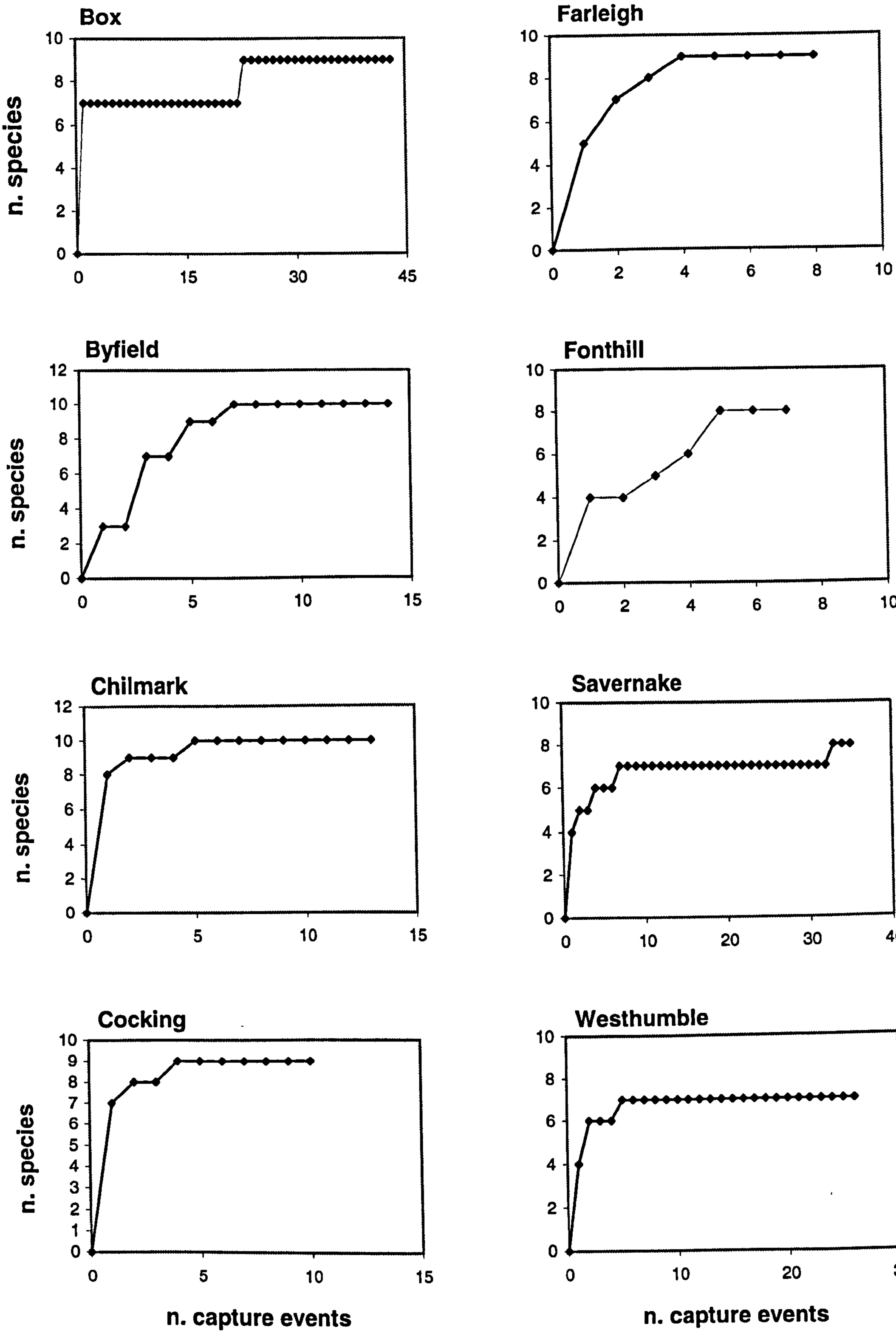


Figure 2.4. Plots showing the relationship between number of species caught and the capture rate of bats, at Box (45 catches), Savernake (28 catches) and at the Tunnels (11 catches).

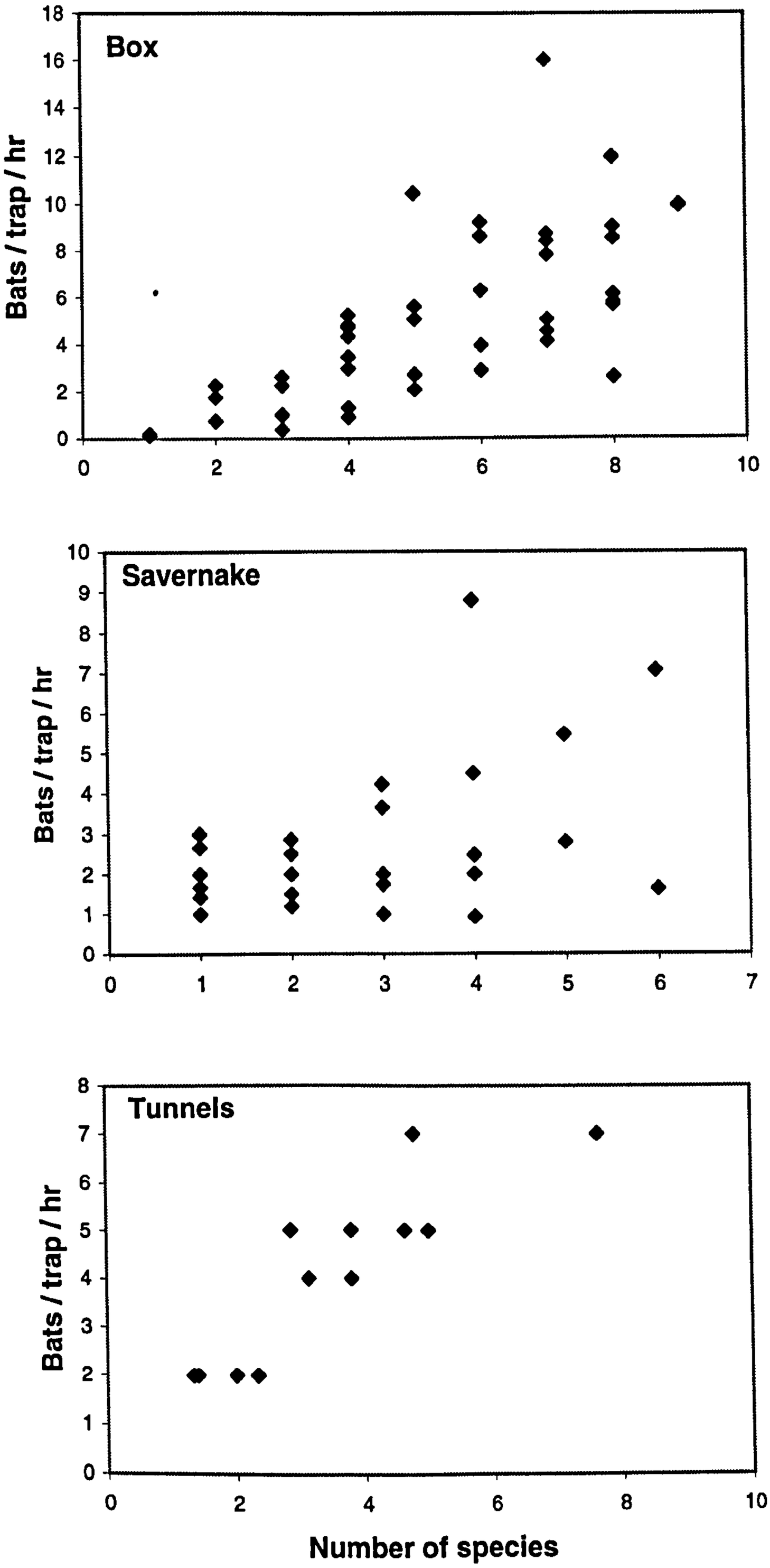


Figure 2.5. Composition of species in autumn (swarming) season (August-November) catches and in spring catches (March-April) pooled for all study sites. n = sample size.

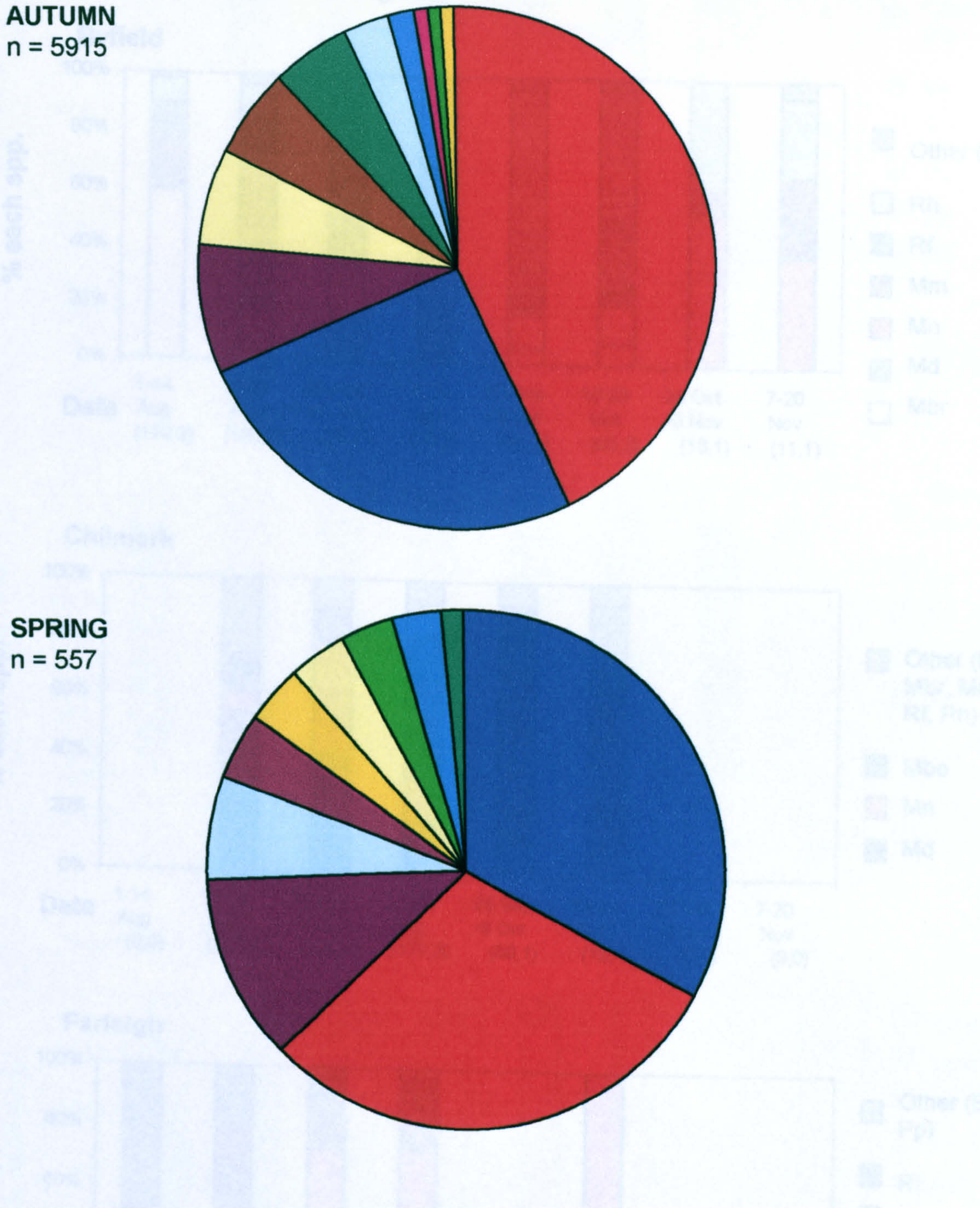


Figure 2.6. Change in species composition with time between 1 August and 20 November for six sites. Data were pooled into fortnightly blocks and average proportions calculated from the catching events within each fortnightly block. Where bars are missing there were no catches within that fortnight block. Species comprising <5% of the total were pooled into category 'Other'. For data from Box see Figure 2.7. Sample size was too small for Fonthill. For species abbreviations please see Fig. 2.5.

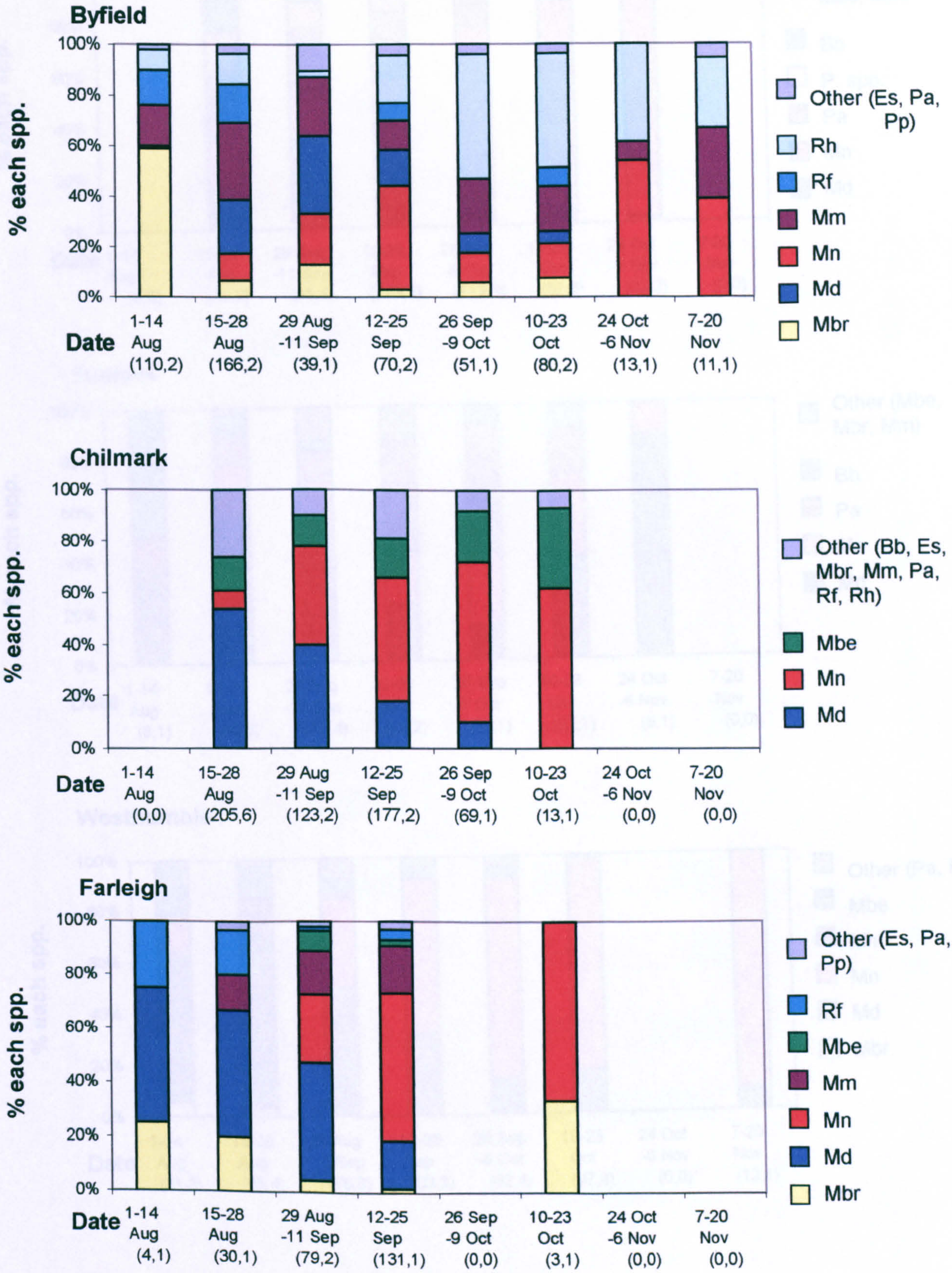


Fig. 2.6. cont.

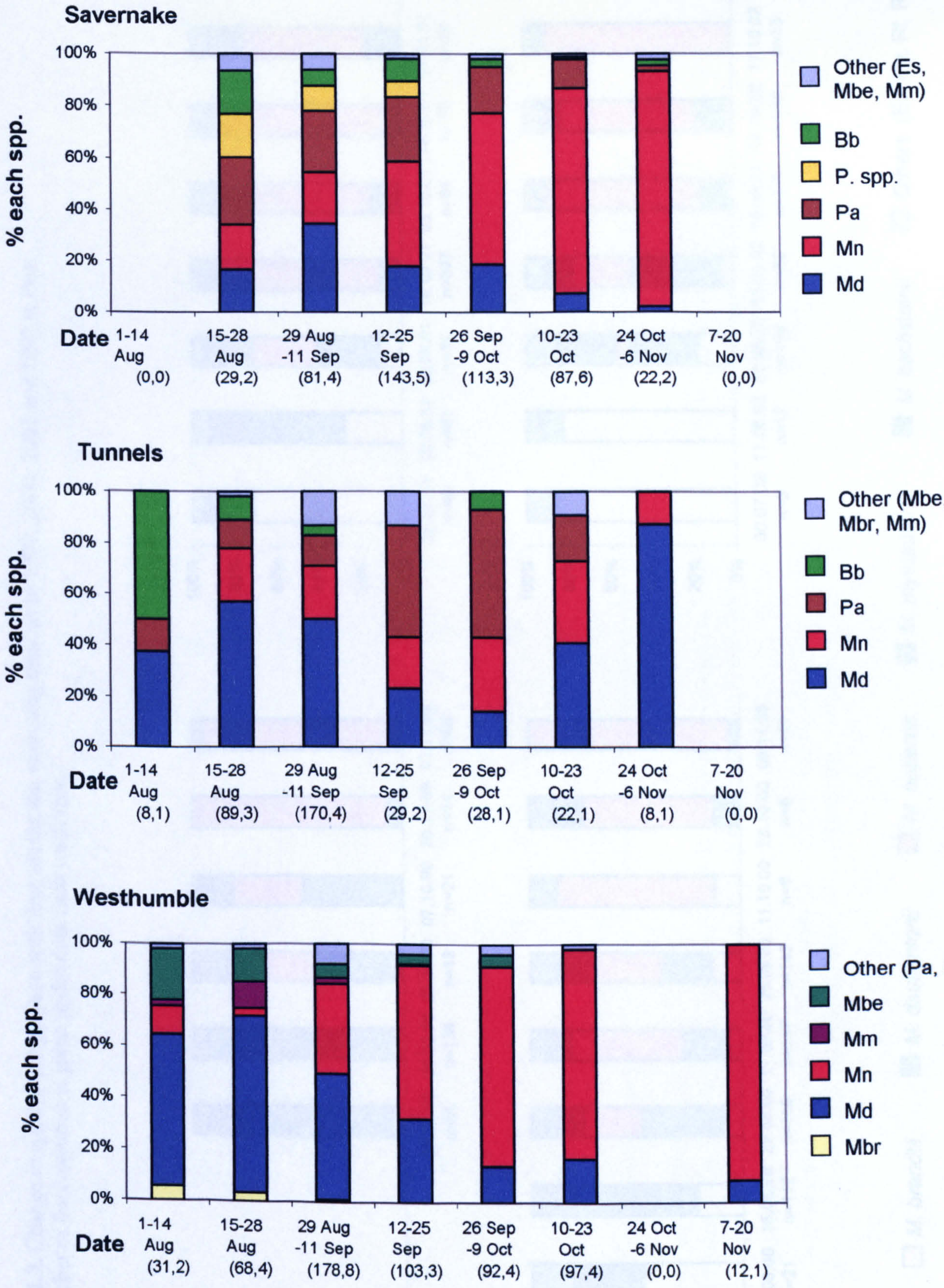

Savernake
100%
80%
60%
40%
20%
0%
% each spp.
Date
1-14 Aug (0,0)
15-28 Aug (29,2)
29 Aug -11 Sep (81,4)
12-25 Sep (143,5)
26 Sep -9 Oct (113,3)
10-23 Oct (87,6)
24 Oct -6 Nov (22,2)
7-20 Nov (0,0)
Other (Es, Mbe, Mm)
Bb
P. spp.
Pa
Mn
Md
Tunnels
100%
80%
60%
40%
20%
0%
% each spp.
Date
1-14 Aug (8,1)
15-28 Aug (89,3)
29 Aug -11 Sep (170,4)
12-25 Sep (29,2)
26 Sep -9 Oct (28,1)
10-23 Oct (22,1)
24 Oct -6 Nov (8,1)
7-20 Nov (0,0)
Other (Mbe, Mbr, Mm)
Bb
Pa
Mn
Md
Westhumble
100%
80%
60%
40%
20%
0%
% each spp.
Date
1-14 Aug (31,2)
15-28 Aug (68,4)
29 Aug -11 Sep (178,8)
12-25 Sep (103,3)
26 Sep -9 Oct (92,4)
10-23 Oct (97,4)
24 Oct -6 Nov (0,0)
7-20 Nov (12,1)
Other (Pa, Rf)
Mbe
Mm
Mn
Md
Mbr

Figure 2.7. Change in species composition with time during the swarming seasons in 1999, 2000, 2001 and 2002 at Box. The number of bats captured is given underneath each catch date.

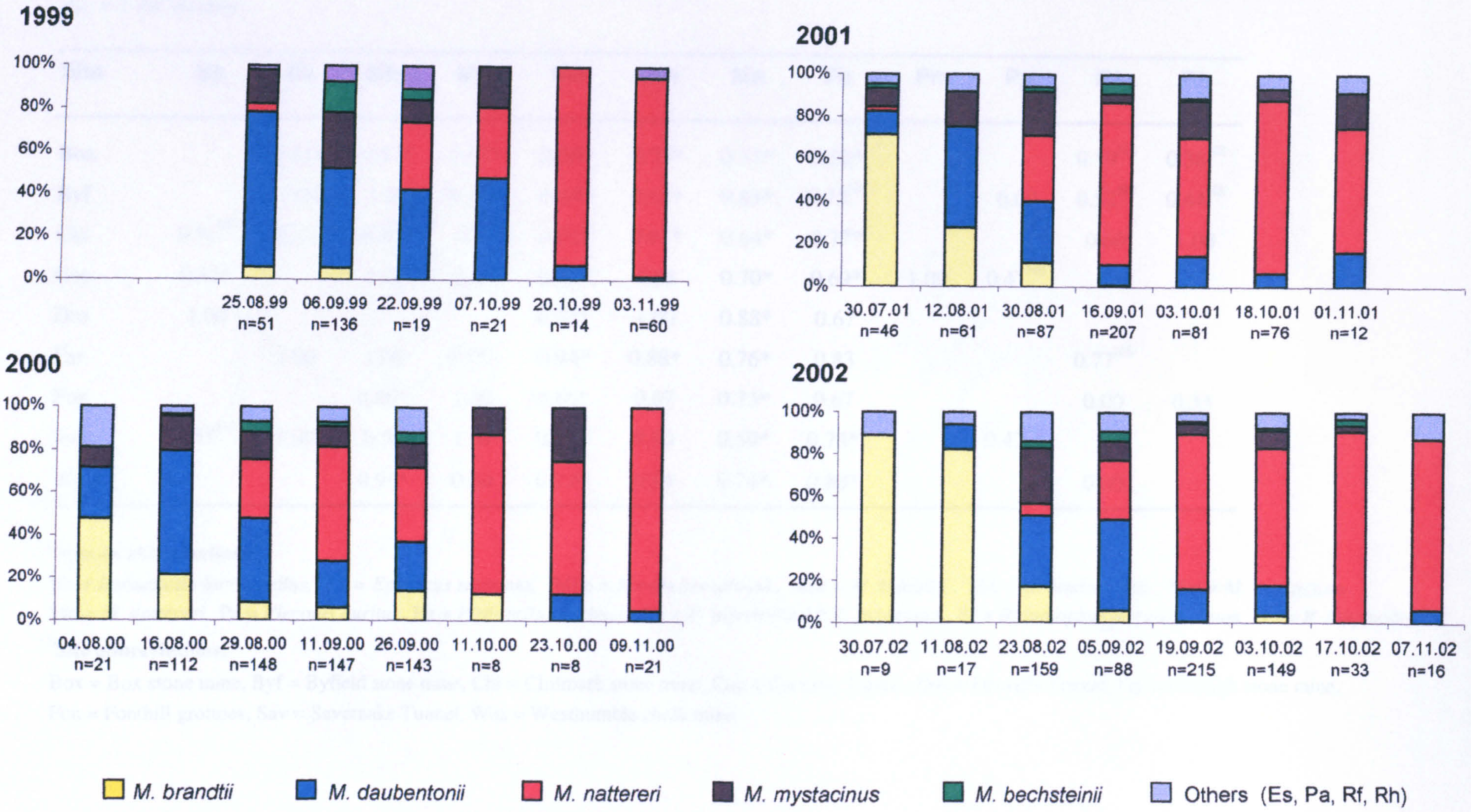


Table 2.2. Sex ratio (n. males captured / n. males and females captured) for each species[a] at each site[b] during swarming season catches. * denotes a sex ratio significantly different from unity ($P<0.05$). ^{NS} denotes a sex ratio not significantly different from unity ($P>0.05$). G-tests were only performed on sample sizes >10. If no symbol (* or ^{NS}) is shown sample size was <10 and G-test was not performed. d.f. = 1 for all tests.

Site	Bb	Es	Mbe	Mbr	Md	Mm	Mn	Pa	Pn	Pp	Rf	Rh
Box		0.81*	0.87*	0.67*	0.78*	0.75*	0.71*	0.82*			0.59^{NS}	0.38^{NS}
Byf		1.00	1.00	0.73*	0.84*	0.81*	0.83*	0.75^{NS}		0.00	0.52^{NS}	0.44^{NS}
Chi	0.75^{NS}	0.75	0.85*	0.71	0.91*	0.91*	0.64*	0.77*			0.60	1.00
Coc	0.81*		1.00	0.33	0.67*	0.88	0.70*	0.69*	1.00	0.47^{NS}		
Dro	1.00				0.74*	1.00	0.88*	0.67				
Far		1.00	1.00	0.75*	0.94*	0.88*	0.76*	0.83			0.77^{NS}	
Fon			0.80*	1.00	0.85*	0.67	0.75*	0.67			0.00	0.33
Sav	0.55^{NS}	1.00	0.50	1.00	0.66*	0.80	0.59*	0.78*		0.47^{NS}		
Wes			0.96*	0.80	0.73*	0.89	0.74*	0.80*			0.00	

[a]Species abbreviations:

Bb = *Barbastella barbastellus,* Es = *Eptesicus serotinus,* Mbe = *Myotis bechsteinii*, Mbr = *M. brandtii*, Md = *M. daubentonii*, Mm = *M. mystacinus* Mn = *M. nattereri,* Pa = *Plecotus auritus*, Pn = *Pipistrellus nathusii*, Pp = *P. pipistrellus & P. pygmaeus*, Rf = *Rhinolophus ferrumequinum*, Rh = *R. hipposideros.*

[b]Site abbreviations:

Box = Box stone mine, Byf = Byfield stone mine, Chi = Chilmark stone mine, Coc = Cocking Tunnel, Dro = Drover's Tunnel, Far = Farleigh stone mine, Fon = Fonthill grottoes, Sav = Savernake Tunnel, Wes = Westhumble chalk mine

Figure 2.8. Change in sex ratio (proportion male in total) (mean ± SD) with time during the swarming season in **(a)** ***M. daubentonii*** and **(b)** ***M. nattereri*** at Box. The number of bats and the number of catches represented by the sample are given in parentheses after the date blocks.

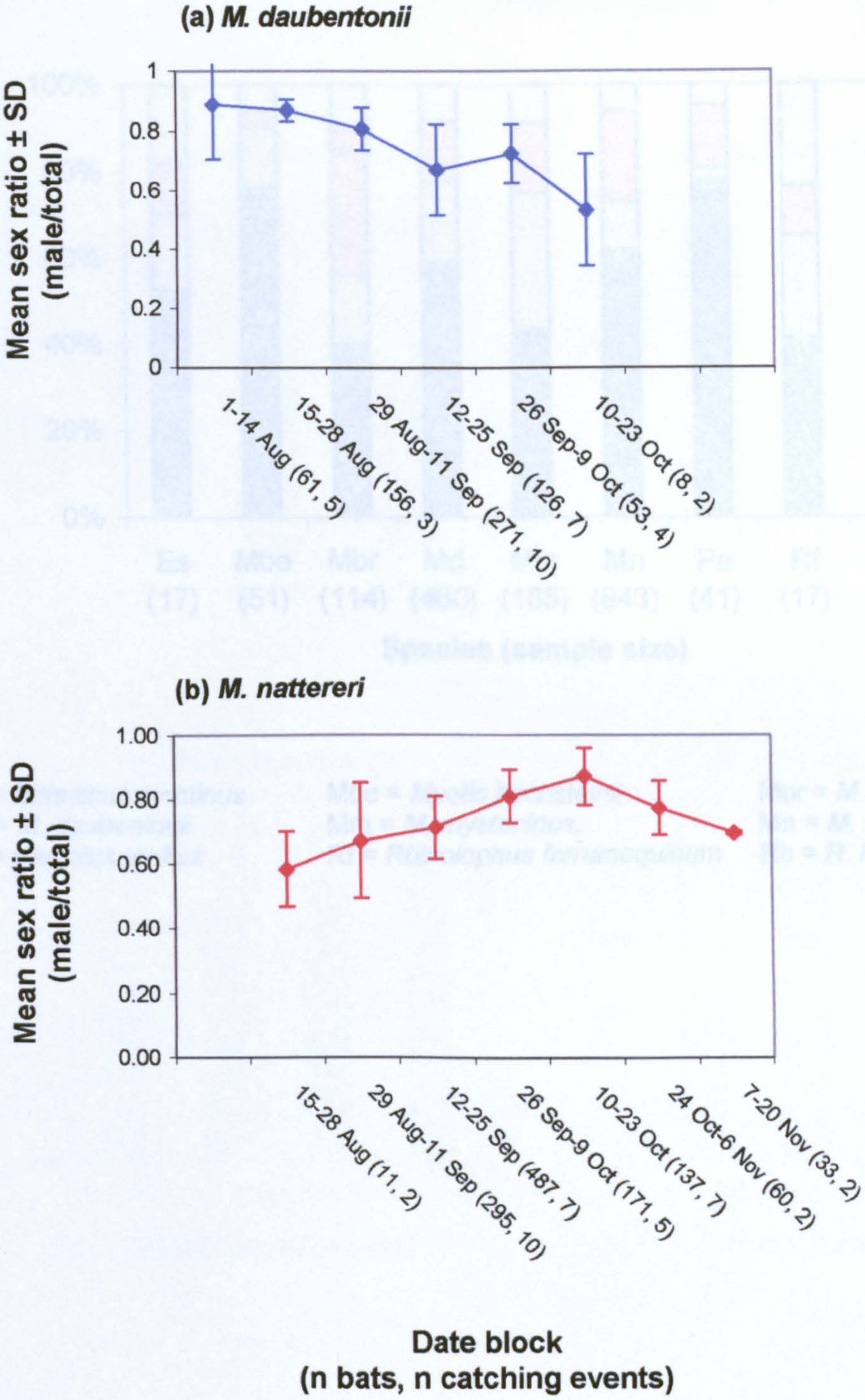
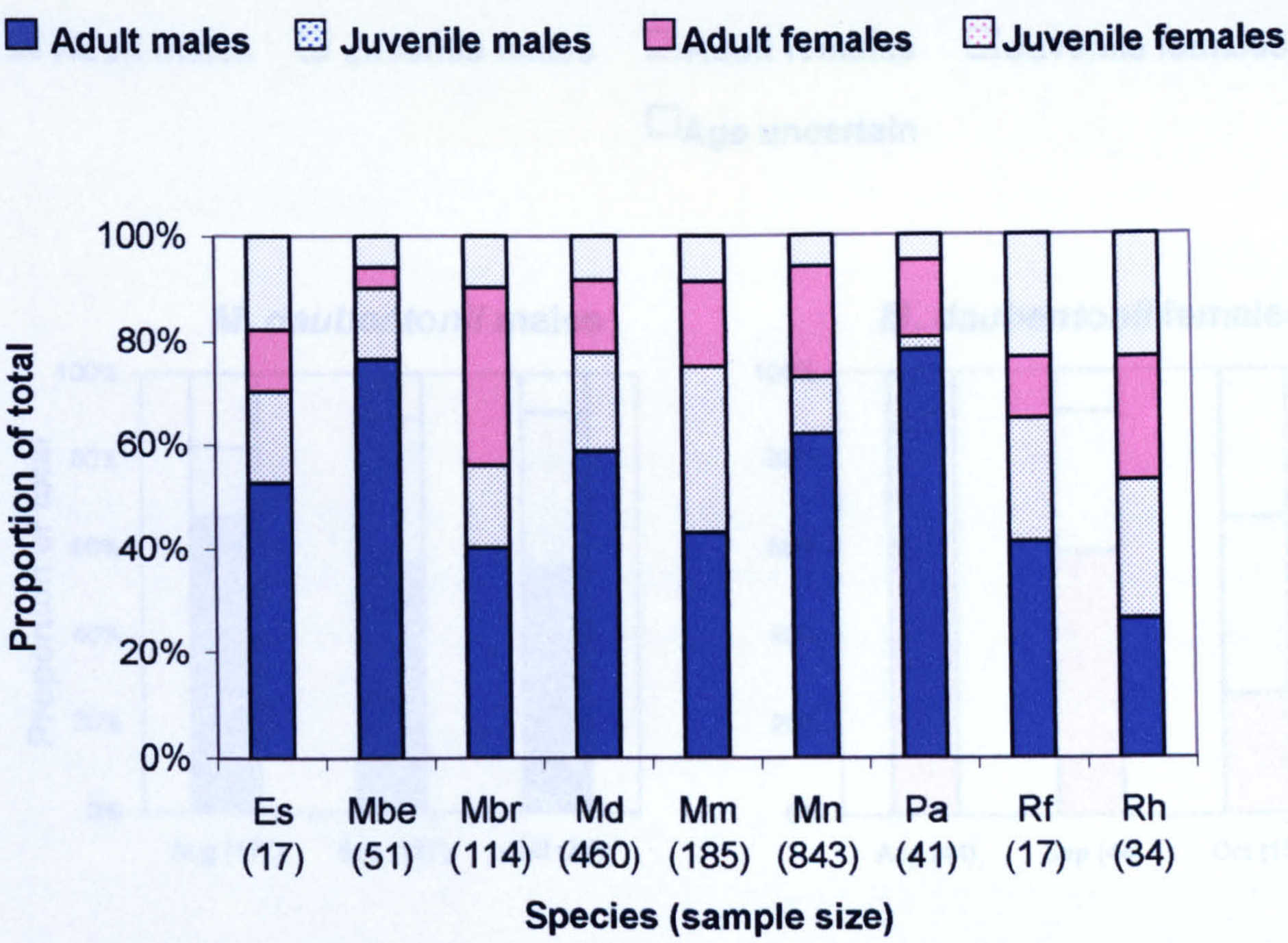
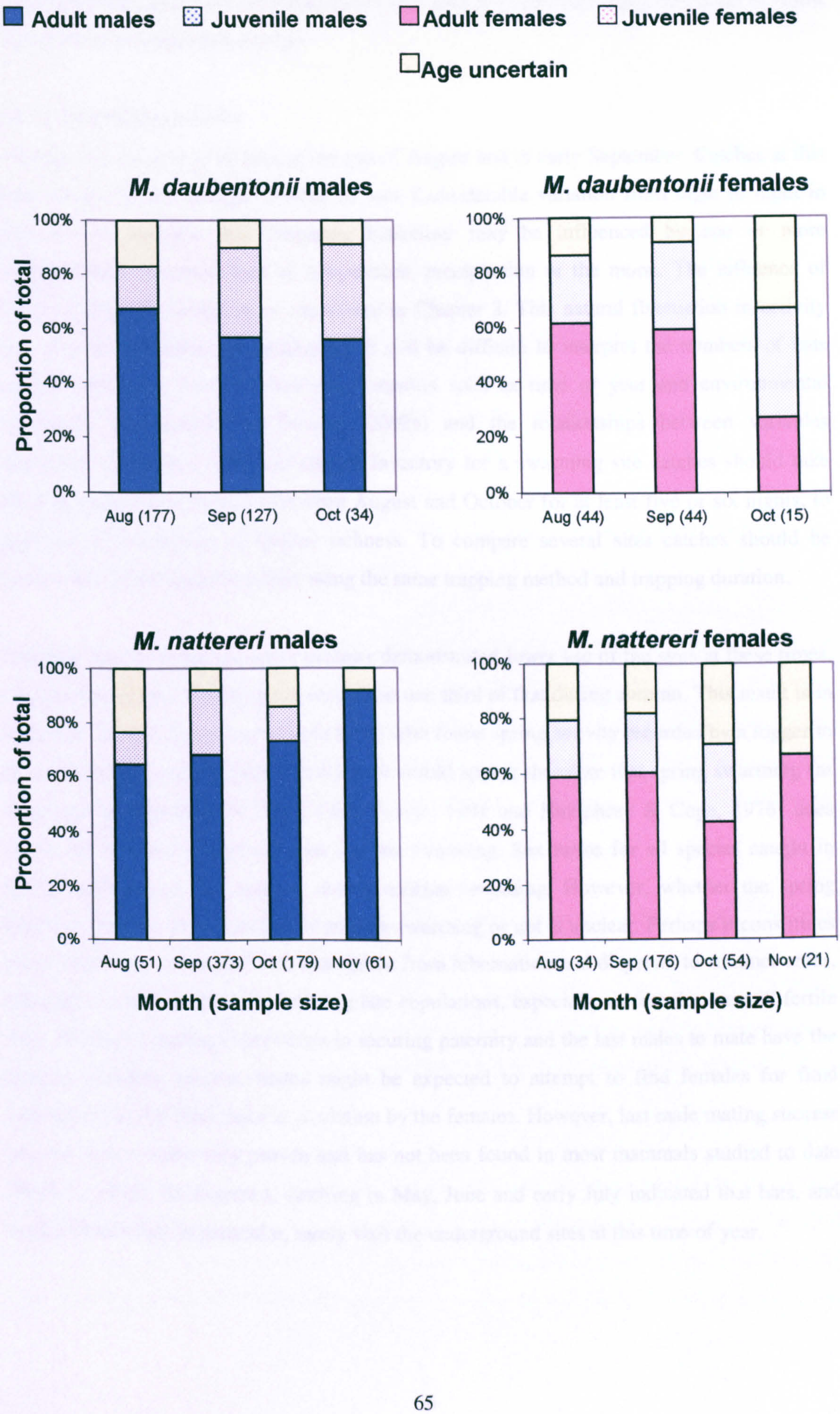


Figure 2.9. Proportions of adult and juvenile males and females of nine species of bat at Box. Number of bats in each sample is given in parentheses after the species abbreviation[1].



[1]Es = *Eptesicus serotinus* Mbe = *Myotis bechsteinii* Mbr = *M. brandtii,*
Md = *M. daubentonii* Mm = *M. mystacinus,* Mn = *M. nattereri,*
Pa = *Plecotus auritus* Rf = *Rhinolophus ferrumequinum* Rh = *R. hipposideros.*

Figure 2.10. Monthly age composition of males and females of *M. daubentonii* and *M. nattereri* at Box. Number of bats in each sample is given in parentheses after the month.



2.5. DISCUSSION

It is evident from these surveys that several of Britain's resident bat species visit underground sites during the autumn for swarming behaviour, similar to that previously described in North America and in continental Europe.

2.5.1. Swarming activity

Activity was greatest at all sites at the end of August and in early September. Catches at this time resulted in the greatest number of bats. Considerable variation from night to night in capture rate indicates that swarming behaviour may be influenced by one or more environmental variables, such as temperature, precipitation or the moon. The influence of these variables on swarming is considered in Chapter 3. This natural fluctuation in activity may present a problem for monitoring. It will be difficult to interpret the numbers of bats present unless the factors influencing variation such as time of year and environmental conditions, are recorded (O'Donnell, 2002a) and the relationships between variables understood. To gain a complete species inventory for a swarming site catches should take place at well-spaced intervals between August and October for at least five or six nights, to approach an asymptote of species richness. To compare several sites catches should be carried out at each simultaneously using the same trapping method and trapping duration.

Catching during spring and early summer demonstrated lower use of the sites at these times. Capture rate of bats during spring was about one third of that during autumn. This result is in agreement with Lubczyk and Nagel (1995) who found spring activity recorded by a logger to be one-third the level of autumn activity. It would appear therefore that spring swarming (as suggested by Bauerová & Zima, 1988; Harrje, 1994 and Humphrey & Cope, 1976) does occur, but at lower magnitude than autumn swarming. Sex ratios for all species caught in spring were almost the same as during autumn swarming. However, whether the spring behaviour has the same function as autumn swarming or not is unclear. Perhaps it constitutes social behaviour connected with emergence from hibernation and dispersal to summer areas, although it could function in obtaining late copulations, especially as females are still fertile then. If order of mating is important in securing paternity and the last males to mate have the greatest breeding success, males might be expected to attempt to find females for final copulations at this time, prior to ovulation by the females. However, last male mating success has not been conclusively proven and has not been found in most mammals studied to date (Hosken, 1998). As expected, catching in May, June and early July indicated that bats, and vespertilionid bats in particular, rarely visit the underground sites at this time of year.

A direct comparison between capture rates at the different sites was not possible because different trapping methods were used and there was nightly variation in activity. Capture rate appeared highest at Box, Westhumble and Farleigh (all stone mines) and least at Fonthill, Chilmark and the Tunnels, but this must be considered with caution. Neither was it possible to compare the number of bats caught during swarming with the number hibernating at a site because hibernation counts are biased towards horseshoe bats which hang from walls and ceilings, whereas *Myotis* species tend to hibernate out of sight (Stebbings, 1988). Most hibernation counts, particularly in mines, grossly underestimate the number of *Myotis* present, supported by observations of *M. daubentonii* by Baagøe *et al.* (1988).

2.5.2. Species composition

In common with studies in North America and in continental Europe the dominant species participating in swarming activity are in the genus *Myotis.* Swarming in Britain is particularly prevalent among *M. nattereri* and *M. daubentonii,* but this could in part reflect their relative abundance compared with the other *Myotis* species (Table 1.2, Harris *et al.,* 1995). More *M. bechsteinii* were caught than would have been expected from previous records and estimated abundance (Hutson, 1993; Harris *et al.,* 1995). The south Wiltshire sites (Fonthill and Chilmark) were particularly rich in this species.

Determining which species are swarming and which visit for another purpose, such as night roosting, is difficult. Frequent capture of *B. barbastellus* during the summer and high recapture rates of marked individuals (F. Greenway, pers. comm.) suggest that this species regularly night-roosts at underground sites (particularly the tunnels, which may also serve as commuting routes) throughout the year. Hence they may not swarm to the degree shown by *Myotis* bats. Similarly, infrequent captures of apparently solitary

E. serotinus may be attributed to night-roosting activity between feeding bouts. Two large maternity roosts of *E. serotinus* are known within 1 km of Box where most *E. serotinus* were captured. In my study *E. serotinus* were not caught beyond mid-August. Bauerová & Zima (1988) also found this species to be an early visitor to swarming sites. Further knowledge is required of the ecology and mating strategies of the less well-known species before determining whether they swarm or not.

More *R. ferrumequinum* and *R. hipposideros* were caught at Byfield than elsewhere, supporting the finding that this site is particularly important as a nursery roost and hibernaculum for these species (R. D. Ransome, pers. comm.). It should be noted that the entrance most frequently used by *R. ferrumequinum* at Box was the least frequently surveyed

by us to minimize disturbance. Therefore this species is probably under-represented at that site.

The apparent abundance of certain species and hence the species diversity scores for the different sites must in part depend on the distribution of catches during the swarming period. Due to the observed temporal segregation in peak activity of *M. daubentonii* and *M. nattereri,* a greater amount of catching later in the season would result in a larger proportion of *M. nattereri* being caught and an overall lower level of species diversity, therefore diversity scores for the different sites are not comparable.

Temporal progression in dominance of *M. daubentonii* to *M. nattereri* during the season has been found by other researchers (Bilo *et al.,* 1989; Harrje, 1994; Lubczyk & Nagel, 1995; Trappmann, 1997). However the peak in *M. brandtii* before that of *M. daubentonii* has not previously been referenced. The order of peak swarming activity is an exact reverse of the pattern seen on departure from hibernation, whereby *M. nattereri* depart earliest followed by *M. daubentonii* and *M. brandtii* are the last to leave (Degn, 1987a). This is probably reflective of the availability of prey and foraging mechanisms of the bats (Barclay, 1991). For example, *M. nattereri* is a gleaning species (Swift & Racey, 2002) and may be better able to locate prey later or earlier in the year than an aerial hawking species like *M. daubentonii* (Turner *et al.,* 2002).

In late July and early August (30 July to 07 August) catching revealed emerging *M. brandtii* at both Box and Byfield. The first bats were caught half an hour before civil sunset. Most of those caught had rock-dust and dirt on their forearms or fur, taken as indication that they had been roosting in crevices in the walls or ceilings or between the 'deads' piled along the walls of the mine, as seen for *M. daubentonii* by Roer and Egsbaek (1966). These captures consisted predominantly of adult males and non-breeding females hence adult breeding females and their offspring were probably still roosting away from the mine at this time. Juvenile *M. daubentonii* and adult *M. nattereri* were also sometimes observed with rock dust on their forearms. Rock dust, particularly later in the season, could have been picked up during mating on the rock surface.

Sampling bias might have influenced the numbers of bats of each species caught. Some species may be better able to detect and avoid traps and mist nets than others. Also, traps and nets may have different degrees of detectability by different species. The efficiency of mist nets and harp traps is not likely to be equal. Preliminary investigations have shown that the two *Rhinolophus* species may be better at detecting and avoiding traps than *Myotis* due to

their higher echolocation frequency and greater maneuverability (Gaisler & Chytil, 2002; S. Dellar, K. Lipscombe & T. McSweeny. pers. comm.). Thus my results may under-represent the number of *R. ferrumequinum* and *R. hipposideros* present. Further investigation into the responses of different species to traps is required.

2.5.3. Sex ratios of swarming bats

A strong male bias was seen in some species (*Myotis* spp. and *Plecotus* spp.), but not in others (*Rhinolophus* spp. and *Pipistrellus* spp.). The former are considered swarming species, but the latter probably visit the site for other reasons, such as night roosting or because they are resident during the day.

Sex ratio in bats is generally unity at birth (see examples cited in Milligan & Brigham, 1993; O'Donnell, 2002c; Rakhmatulina, 1995; Ransome & McOwat, 1994). Rakhmatulina (1995) later states that in temperate regions sex ratios are often shifted in favour of males, but whether this is at birth or later in life is unclear. Davis and Hitchcock (1964) found populations of *M. lucifugus* and *E. fuscus* hibernating in a mine were 72% and 70% respectively biased towards males, but in Indiana Mumford (1958) found 55% males among hibernating *E. fuscus*. For the species considered here, Harrje (1994) found 47% males in hibernating *M. daubentonii* and Stebbings (1965) found 59% males in hibernating *M. nattereri*. On departure from hibernation sex ratio of *M. daubentonii* was 1:1.1 ♂:♀ (Baagøe *et al.*, 1988). Sex ratio was still male-biased for the four most abundant species of *Myotis* during spring in my study. This contrasts with findings by Furmankiewicz & Górniak (2002) who found sex ratios at unity for *M. mystacinus* and *M. nattereri* during spring.

Based on the available literature it therefore seems likely that the sex ratio observed during swarming (for swarming species) is not reflective of the actual sex ratio in the population, which is likely to be closer to unity (e.g. sex ratio of new born *M. daubentonii* was approximately 1:1 by Kurskov, 1981 cited in Jurczyszyn & Bajaczyk, 2001). The finding that sex ratio is more male-biased in swarming species than in non-swarming ones lends support to the mating strategy hypothesis of swarming. Large numbers of males may gather to wait for females that visit less regularly and therefore appear less abundant. The mating strategy is different for *Rhinolophus* spp., in which solitary males occupy territories for a period of days/weeks during which time females visit and select a male for mating (Ransome, 1990), and *Pipistrellus pipistrellus*, in which solitary males defend mating roosts and advertise for females (Gerell & Lundberg, 1985). In this country rhinolophids and pipistrelles might visit swarming sites for day-roosting, night-roosting or a social behaviour that is equally attended by males and females. *R. ferrumequinum* have breeding colonies at Box and Byfield and both

species are seen frequently in hibernation. These are the only species to roost consistently underground at the sites, which could explain the equal sex ratio. Invasion behaviour, seen in pipistrelles in Germany (Grummt & Haensel, 1966; Smit-Viergutz & Simon, 2000), has not been documented for pipistrelles in Britain and was not found in my study.

Although sex ratio changed with time during the swarming season the change was not consistent. In *M. daubentonii* sex ratio approached unity by mid-October perhaps indicating that females arrived and swarmed later than males. Populations of *M. nattereri* became progressively more male biased toward mid-October and then less male-biased to mid-November, perhaps indicative of late arrival of females in this species also. Daan (1973) stated that female *M. daubentonii, M. dasycneme* and *M. mystacinus* tended to arrive and start hibernation earlier than males, which is therefore in contrast to my findings.

2.5.4. Age composition

In all species caught during swarming some individuals were identified as juveniles. Sex ratio was still male-biased among juvenile *Myotis,* in contrast to findings by Schowalter (1980) and Fenton (1969) for recaptured marked bats. The observation that proportion of juvenile *M. daubentonii* increased toward the end of swarming supports observations that juvenile bats enter hibernation later than adults (Schowalter, 1980). No attempts were made to age bats during spring, however Baagøe *et al.* (1988) found that an x-ray method worked effectively at this time, which could perhaps be incorporated into future studies.

2.5.5. Implications for conservation

Surveys at swarming sites have revealed far more species and individuals than would otherwise be recorded. Underground sites have been known to be important refuges for British bat species during breeding and hibernation. Such sites appear to have equal or perhaps greater importance during late summer and autumn as sites for swarming and for night-roosting for a large variety of bat species including, in Britain, all five resident species of *Myotis, P. auritus, B. barbastellus* and *E. serotinus.*

The UK population of *M. nattereri* may be of international importance (Hutson, 1993; Macdonald & Tattersall, 2001; Stebbings, 1988) and this species was found frequently at all sites together with *M. daubentonii.* My survey indicates that *M. bechsteinii* and *B. barbastellus,* species considered rare at a national level (Hutson, 1993; Macdonald & Tattersall, 2001), may focus at underground sites from large areas and hence protection of such sites is vital. Sites of particular use at certain times of year by these vulnerable species must be given special protection.

Bat detector surveys and hibernation counts alone are not adequate to assay the numbers of species and individuals present in an area or at a particular hibernation site, particularly for species with low population densities. Consequently the conservation importance of such sites might be grossly under-estimated. For example, a small mine system in Germany was due for destruction by in-filling because it was thought to be an unimportant wintering site (Kretzschmar, 1994). Swarming catches identified the importance of the site for the local bat population and consequently it was saved from destruction.

Swarming season surveys of underground sites should be included in population studies in an area to give a more complete picture of the species richness and hence the importance of sites with respect to planning by conservation authorities. Due to the high number of species and individuals at swarming sites criteria should be developed for selecting SSSIs. In the UK the majority of bat SSSIs are designated for roosting bats, hence swarming in which the majority of bats are not (permanently) resident at the site adds a new dimension to bat conservation. That some of the sites are also important hibernacula for *R. ferrumequinum* and *R. hipposideros,* and in some cases also nursery sites, bodes well for their protection, but the high activity between August and October must be taken into account in conservation planning. Consideration must be given for example when giving permission for caving activities that frequently take place in the evening after dark. While conducting one catching survey a group of cavers arrived and asked to be allowed past our traps to enter the system. Such activities could cause disturbance to the swarming bat community. Observations have shown that most activity during swarming occurs in and around the entrances to underground sites; hence the design and placing of grilles may be of importance in the continued use of such sites by bats.

Catching is an invasive technique (discussed in Chapters 3 and 4) and should be used only when absolutely necessary. The development of non-intrusive, automated multi-species monitoring systems with consistent recording protocols should be a priority (see Chapter 3).

Conservation of swarming sites is especially important if the sites are confirmed to be important centers for mating activity as suspected. There may be a limited number of suitable sites in the region, and if site fidelity is high then site loss would have important consequences for the survival of local and perhaps regional populations. Discovering the sizes of populations of swarming species (Chapter 4) and the catchment area from which bats are drawn to swarming sites (Chapter 5) may help in our understanding of the interactions between bats from different colonies at swarming sites and may aid estimates of national population sizes.

CHAPTER THREE

EFFECTS OF SEASON, WEATHER CONDITIONS AND TIME OF NIGHT ON SWARMING ACTIVITY

3. EFFECTS OF SEASON, WEATHER CONDITIONS AND TIME OF NIGHT ON SWARMING ACTIVITY [1]

SUMMARY

Bat activity at Westhumble chalk mine was recorded automatically with a frequency division logging system on 415 nights over five years (1997 to 2001)*. Nightly activity was highest between the beginning of August and the end of October, with a peak in September. This pattern was consistent across years. Bat activity was recorded automatically at Byfield mine system on one night per month between August and October over three years (2000 to 2002)**.

Activity varied markedly from night to night and was affected by rainfall (which significantly suppressed swarming activity), and maximum temperature (with which activity was positively correlated). Moon phase had no detectable influence on swarming activity. From this I conclude that bats are most likely to make the journey to swarm when weather conditions are favourable (warm and dry) and when they have been able to forage sufficiently in advance of the journey to attain a positive energy budget.

The nightly pattern of activity was studied at Westhumble between 1998 and 2001 and at Byfield limestone mine in 2000, 2001 and 2002. Activity was low in the first few hours after sunset of each night during the swarming period indicating that there was low daytime occupancy of the site. Activity increased to a peak between five and seven hours post-sunset consistent with a large number of bats arriving after the first evening foraging spell. Activity then decreased gradually to dawn as these bats departed again. This pattern was consistent throughout the swarming season. Logging at Byfield revealed that most bat activity was concentrated near to the entrances, and few bats visited the center of the mine system.

Logged activity positively correlated with the number of bats caught per hour at Westhumble, confirming that loggers are a reliable alternative to catching when monitoring a swarming site. Development of loggers able to distinguish between species would be advantageous to research and conservation work.

* Field work carried out by F. Greenaway. ** Field work carried out by R.D. Ransome.

[1] A paper based on the Westhumble data presented in this chapter has been accepted for publication in Journal of Zoology under the title 'Swarming activity of temperate zone microchiropteran bats: effects of season, time of night and weather conditions'. G. Jones and F. Greenaway are co-authors.
See Appendix 2 for copy of proof.

3.1. INTRODUCTION

On some nights many more bats may visit a site to swarm than on others (Harrje, 1994; Humphrey & Cope, 1976; see also Chapter 2) and this variation in visitation rate may be related to environmental factors. Humphrey and Cope (1976) reported that levels of activity were synchronous at two nearby sites, suggesting that swarming was influenced by a factor affecting an area in a uniform manner, such as prevailing weather conditions or moon phase.

3.1.1. Bat activity and the weather

Activity of free-flying foraging bats is positively correlated with ambient temperature (Erickson & West, 2002; Gaisler *et al.*, 1998; Vaughan *et al.*, 1997; Walsh & Harris, 1996) most likely because insect abundance is greater at higher temperatures (Jones *et al.*, 1995; Rydell, 1989; Williams, 1961). Park *et al.* (1999) recorded increased activity of *Rhinolophus ferrumequinum* at higher cave temperatures during the hibernation period. Swarming activity of *Pipistrellus pipistrellus* in a castle cellar, between May and September, was positively correlated with ambient temperature and was depressed by high wind speeds (Sendor, 2002). Erickson & West (2002) found a negative association of bat foraging activity with rain and Fenton (1969) stated that rain invariably caused a decline in the numbers of bats visiting a mine during swarming. Rain might reduce bat activity either because it suppresses the activity of insects, or because it is disruptive to bats' flight or echolocation ability.

I hypothesise that bats will travel to swarming sites when weather conditions are favourable for long-distance journeys and when foraging is good, so that they have a positive energy budget on the outset of their journey, while at the swarming site, and at the end of the night for the return journey. I predict therefore that swarming activity of (predominantly) *Myotis* species at a swarming site/hibernaculum will be positively correlated with ambient temperature and negatively correlated with rainfall.

3.1.2. Bat activity and the moon

In general temperate zone insectivorous bats are not lunar-phobic when foraging (Gaisler *et al.*, 1998; Hayes, 1997; Negraeff & Brigham, 1995; Vaughan *et al.*, 1997) unlike some tropical frugivorous species (Elangovan & Marimuthu, 2001; Morrison, 1978; Nair *et al.*, 1998), possibly because temperate species experience less predation risk than tropical species. However, some insectivorous species modify their behaviour on brightly moonlit nights by flying in shadows, closer to vegetation or at different heights in the forest canopy (Fenton *et al.*, 1977; Hecker & Brigham, 1999; Reith, 1982). Karlsson *et al.* (2002) found that moon phase did not affect the number of bats flying outside or inside a mine during swarming.

I hypothesise that the moon might have two opposing effects on swarming activity. Firstly, activity might be inhibited on bright nights because bats swarming at the entrances to underground sites are more visible to visual predators, for example hawks that might wait at the entrances. In addition, bats may experience greater predation risk while on their commuting journeys from roost to swarming site on a brightly lit night than when there is no moon. However, if the use of stellar cues was important in navigation bats might be expected to make journeys to swarming sites on dark, cloudless nights when stars are most visible. Conversely, if visual landmark cues were required for long commuting journeys swarming activity might actually be enhanced on brightly moonlit nights.

3.1.3. Automatic logging of activity

Automatic logging has been used to monitor bat activity levels in several studies. Sendor *et al.* (2000) studied activity at a hibernaculum of *Pipistrellus pipistrellus* by using infra-red light barriers and ultrasound sensors. Degn *et al.* (1995) used light barriers to study activity of *Myotis* bats (mostly *M. daubentonii*) at a limestone mine throughout the year. Both researchers commented on swarming activity though it was not the main focus of the papers. Lubczyk & Nagel (1995) also used a light barrier but incorporated a camera to identify bats to species as they flew through the entrance to a swarming site. They found that activity of bats was three times higher during autumn than in spring and photo-identification allowed them to delineate the period of activity of *M. daubentonii* from that of *M. nattereri* (see Chapter 2).

The benefits of using automatic loggers to monitor bat activity include lack of disturbance to the bats being studied, particularly when compared to catching or hibernation counts (Thomas, 1995) and ease of data collection. Once a logging system is in place it may be left for a length of time, potentially months, before data are downloaded, and a large quantity of information can be gained with small input of time and effort. The systems can also be relatively inexpensive, particularly if custom-built.

The main disadvantage is the difficulty in discriminating between species. Even a sophisticated logger that records time-expanded echolocation calls for analysis cannot, with present technology, discriminate with a high degree of confidence between the five species of *Myotis* resident in Britain (Parsons & Jones, 2000; Vaughan *et al.*, 1997). A photo-identification system such as that used by Lubczyk and Nagel (1995) is only useful where the bats' flight is constrained through a small opening where the photograph can be taken and when species are easily identifiable from one another. In my study, *M. mystacinus* and *M. brandtii* can only be distinguished from one another in the hand and may be confused with *M.*

daubentonii in a photograph. A logging system will be of benefit in long-term monitoring only if it is actually representative of the number of bats present. This can be tested by simultaneously carrying out catching surveys, which also presents an opportunity to test whether catching does actually have a negative impact on bat activity.

The specific aims of this chapter are:

1. To document activity at a hibernaculum during the course of the year to establish when swarming occurs.
2. To discover whether activity during the swarming period covaries with temperature, rainfall or the phases of the moon.
3. To identify changes in activity during the night as the swarming period progresses.
4. To find out what regions of a mine bats visit during swarming.
5. To test whether bat activity recorded automatically correlates with numbers of bats caught and hence can be a reliable in monitoring the population visiting the site.
6. To ascertain whether catching has a negative influence on bat activity.

3.2. METHODS

3.2.1. Logging equipment

At Westhumble[1] an activity logger was situated 15 m inside the grilled entrance (Plate 3.1). The equipment comprised a custom-made frequency division bat detector monitoring echolocation call frequencies of all bat species in the study area connected via a Schmitt trigger to a datalogger (Tiny Tag, Gemini Dataloggers), which logged the number of bat passes (distinct series of calls separated by a period of quiet consistent with a pass by one bat, Fenton, 1970) per hour (per night in 1997). The gain of the bat detector was set to the same level at all times to keep the sensitivity constant. After each bat pass the unit was deactivated for ten seconds. All bat species were therefore detected by the system and repeat triggers by a single bat pass were avoided. No distinction was made between the different species present. However, from catching surveys (Chapter 2) 97% were of the *Myotis* genus. Activity data were downloaded onto a laptop computer using the program Otlm (version 1.4, Gemini Dataloggers). The logger was run for a total of 415 days between 1997 and 2001, including at least part of the swarming season (August to November) each year. Data were occasionally lost due to equipment malfunction and flooding of the mine. The equipment was rebuilt following flooding at the end of 2000.

At Byfield[2] in 2000, activity loggers comprising a Tranquility II bat detector (David Bale) set to time-expand calls by 32 times linked to a voice-activated dictation machine and time calibrated by a talking clock were used. In 2001 and 2002 Eco Mega detectors (David Bale) were used which had a built-in timer and digital memory to store calls. The benefit of the Eco Mega system over the Tranquility II was the facility to reject triggers from water droplets. The logging units were situated at various locations in the mine system (Fig. 3.1) on one night per month between August and October. Each discrete echolocation call recorded was analysed by looking at its call parameters (frequency, duration) to determine the species that produced it. Calls were separated into those of *R. ferrumequinum*, *R. hipposideros* and vespertilionid bats (*Myotis* spp. and *Plecotus* spp. but almost exclusively the former. Catching revealed 97% and 3% respectively – see Chapter 2). The calls of vespertilionid bats are too similar to be able to distinguish species by this method of analysis. The numbers of calls of each type were recorded per hour for each location on each night and summed to provide nightly totals. Only those of vespertilionid bats are considered here.

[1] The activity logger at Westhumble was constructed, maintained and downloaded by Frank Greenaway, who has kindly allowed me access to his data for analysis. Frank also carried out catching at the site.

[2] The activity loggers at Byfield were constructed, maintained and calls analysed to family by Roger Ransome (Bat Pro Ltd.) on behalf of Bath and North East Somerset Council who have kindly allowed me access to their data for analysis.

3.2.2. Environmental variables

Maximum and minimum temperatures (°C) and rainfall (mm) per 24 hours (12pm to 12pm) were obtained from a weather station located 1 km away from the mine entrance at Westhumble. Percentage of moon face illuminated and times of official sunset and sunrise were obtained from Whitaker's Almanack (Anon, 1997; Hill, 2000; Hill, 2001).

3.2.3. Capture of bats

Bats were caught for general survey purposes on sixteen occasions during August, September and October at Westhumble (Chapter 2) while the activity logger was operational. Capture was by a single custom-made harp trap placed at 1 of 2 locations within the mine (12 m and 25 m in from the entrance) and at the entrance on a single occasion. Placement of the trap was identical each time a particular location within the mine was used. Trapping started around dusk and normally continued until after midnight unless curtailed by rain or on one occasion to follow radio-tagged animals.

3.2.4. Data analysis

For analysis of annual activity at Westhumble the number of bat passes recorded during hours of darkness (official sunset to official sunrise) were summed for each date of recording to produce a nightly activity index.

To remove the variation in activity caused by the changing seasons and to concentrate on variation in activity from day to day, curves were fitted to the observed activity data from 1 August to 31 October in 1997, 2000 and 2001. The curve that most closely described the data (greatest correlation coefficient, *r*) was selected from those available from the 'peak' equations menu in SigmaPlot (version 5.0). Residuals were calculated from the 'observed' and 'expected' data to describe whether activity was 'higher' or 'lower' than expected.

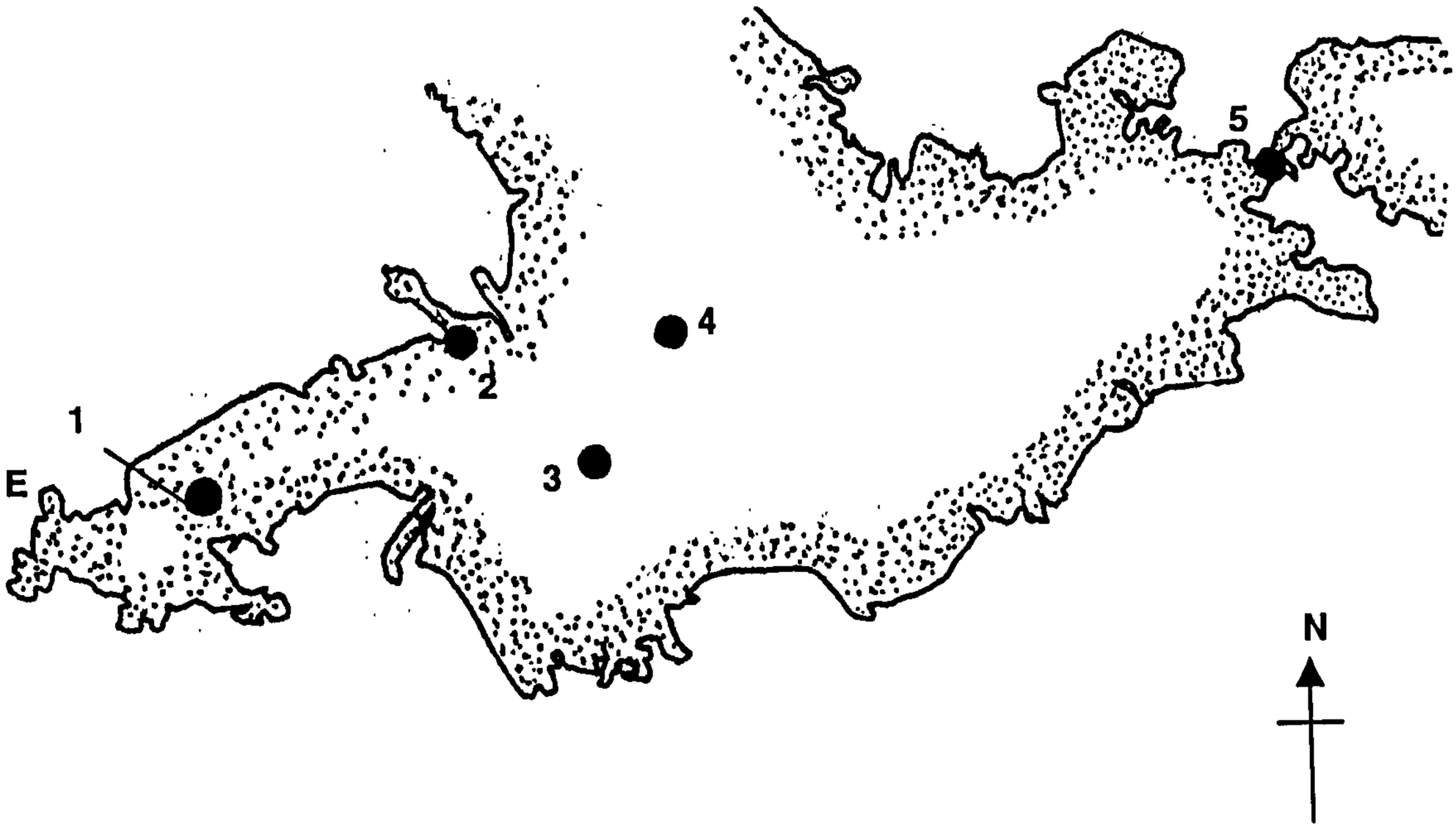
Residuals of maximum and minimum temperature were calculated from linear regression lines fitted to the data to remove seasonal effects, and analyses were performed with the residual temperatures ('warmer' or 'colder' than expected for the time of year). Rainfall and the different phases of the moon were not related to season throughout the swarming period in each year, hence raw data were used in analysis of these variables.

The number of bats caught in the harp trap was divided by the number of hours of catching on each occasion to produce a measure of bats caught per hour in order to standardize bats present per unit time.

Parametric statistics were used where data were transformed (log_{10}) from non-normal to normal (tested in Minitab version 11); otherwise non-parametric statistics were used (Zar, 1999; Siegel & Castellan, 1988). In the correlations between residual activity and each environmental variable $\alpha = 0.0125$ because a Bonferroni adjustment was applied to the critical value of α to minimise the chance of Type 1 error.

Plate 3.1. Westhumble chalk mine. The building at right protects the grilled entrance to the mine and provides an additional refuge for bats.

Figure 3.1. Map of the mine at Byfield showing the location of automatic logging units (not to scale but see distances given below in the key).



KEY:

E = entrance	
1 = logger unit at 'pillars'	30 m inside entrance
2 = logger unit at 'Sector X'	200 m inside entrance
3 = logger unit at 'Irvine's'	300 m inside entrance
4 = logger unit at 'watering can'	300 m inside entrance
5 = logger unit at 'Byfield-Firs connection'	800 m inside entrance

3.3. RESULTS

3.3.1. Annual activity at Westhumble

Most activity occurred between the beginning of August and the end of October, peaking in September (Fig. 3.2). Moderate activity continued through November and December and activity during the remainder of the year was low. This pattern was consistent across years (Fig. 3.3), but activity during 2001 (August to October) was significantly greater on average than for previous years (Kruskal-Wallis: H = 86.5, d.f. = 4, $P < 0.0001$). Multiple comparisons following the Kruskal-Wallis test showed that 2001 was significantly different to all other years and 1999 differed from 2000. Great variation occurred in activity levels from night to night (Figs. 3.2 & 3.3).

3.3.2. Correlation of activity with environmental variables

Residual nightly activity during the swarming season (1 August to 31 October inclusive) in years 1997, 2000 and 2001 was correlated against a range of environmental predictors. Figure 3.4 shows the curves fitted to the data from which residuals were calculated for analysis.

Residual activity was significantly negatively correlated with rainfall (Fig. 3.5a) (Spearman's Rank correlation coefficient (r) = -0.335, d.f. = 270, $P < 0.0001$). On days with high rainfall activity was lower than expected. Rainfall particularly suppressed swarming activity at levels exceeding 15 mm per 24-hour period (Fig. 3.5a). Residual activity was positively correlated with residual maximum temperature (Fig. 3.5b) (r = 0.189, df. = 270, P = 0.002). In general therefore, bat activity was greater at higher temperatures. At temperatures below 12-13°C bat activity was particularly reduced (Fig. 3.5c).

There was no correlation between residual activity and residual minimum temperature (r = -0.046, d.f =270, $P > 0.20$) (Fig. 3.6a) or between residual activity and moon phase (r = 0.091, d.f. = 270, P > 0.10) (Fig. 3.6b). However, there was a slight trend for lower activity than expected during the lighter phases of the moon (gibbous and full moon) and higher activity than expected during the darker phases (crescent and quarter moon) (Fig. 3.6c). The result for new moon appears anomalous because if bats were exhibiting lunar phobia, the highest activity score would be expected during this phase when the night sky is darkest.

3.3.3. Nightly activity at Westhumble and Byfield

Hourly activity at Westhumble was averaged for half-monthly periods during the swarming season (Fig. 3.7). Activity was recorded in most hours between sunset and sunrise, although it was not evenly distributed throughout the night. Activity was low in all periods for the first

three to four hours following sunset. Activity then increased during the next three hours to reach a peak between five and seven hours post-sunset. This was most marked in the first and second halves of September when activity was greatest. Activity then gradually decreased until dawn. In most months, very low levels of activity were recorded post-sunrise, indicating prolonged activity of a few bats within the mine during the day.

Hourly activity at Byfield indicated a similar pattern to that at Westhumble of low activity for the first three hours after dusk, followed by a sharp increase in activity several hours after dusk and then a gradual decrease in activity to dawn (Fig. 3.8). The number of calls decreased with distance from the entrance to the system indicating that the activity is concentrated primarily near the entrance and up to 200 m inside (Fig. 3.9). The number of calls logged was lower at 300 m and very few calls were recorded at 800 m from the entrance.

3.3.4. Logged activity and the capture of bats

The log-transformed number of bat passes recorded by the logger on catch nights (n = 16) was positively correlated with the log-transformed number of bats caught per hour (Fig. 3.10) ($r = 0.617$, d.f. = 14, $P < 0.01$). Numbers of bats caught were no greater during 2001 than in previous years (min 8 to max 33 per catch compared with 6 to 76 in previous years).

There was no significant difference between the numbers of bat passes recorded on catch days (median = 134) and on the days following catching events (median = 129) (Mann-Whitney U test: $W = 244.5$, $N_1 = 16$, $N_2 = 16$, $P = 0.474$). Similarly, there was no significant difference between numbers of bat passes recorded on the days preceding catch days (median = 131) and the catch day itself ($W = 0.01$, $N_1 = 16$, $N_2 = 16$, $P = 0.777$).

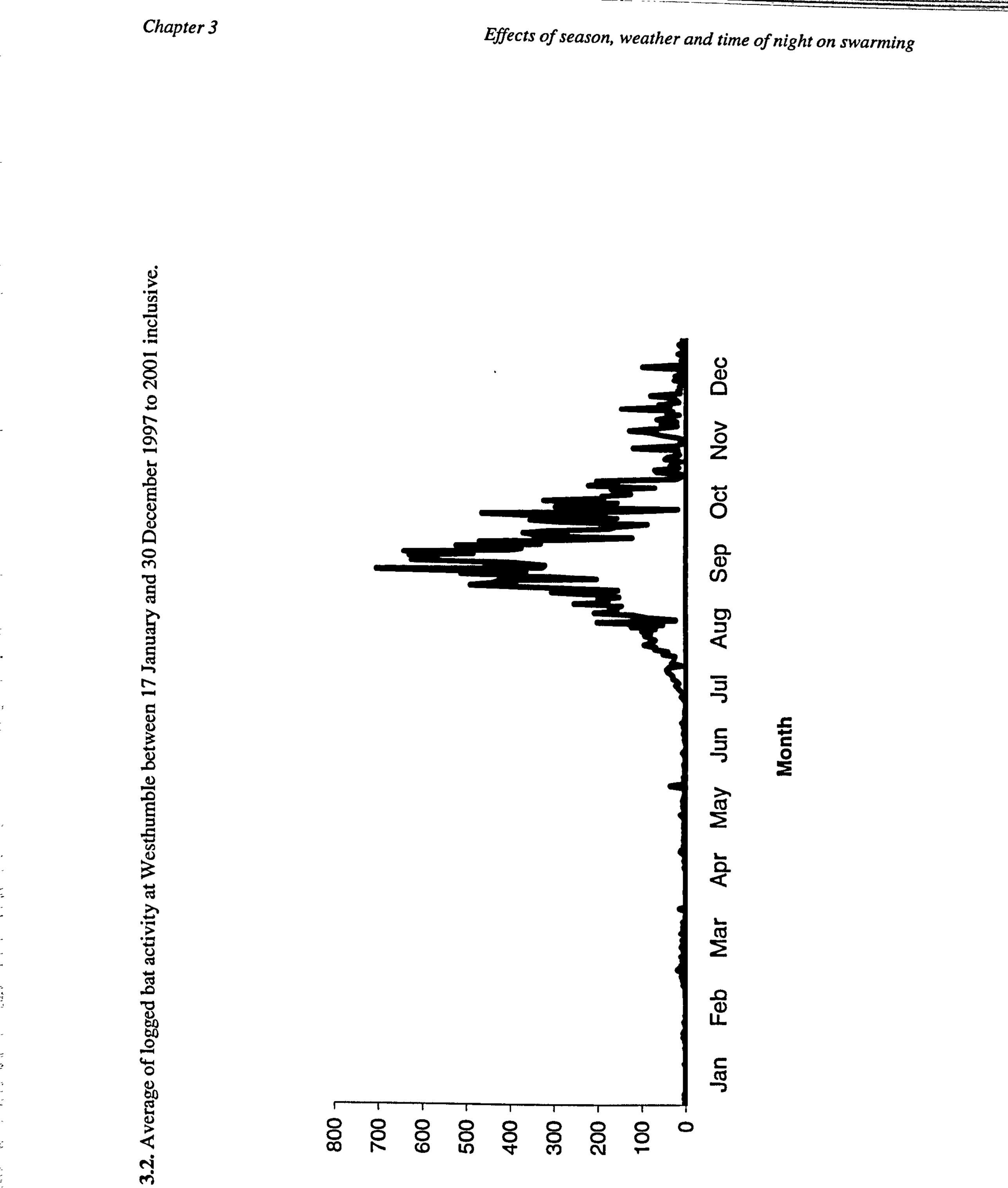


Figure 3.2. Average of logged bat activity at Westhumble between 17 January and 30 December 1997 to 2001 inclusive.


Year2001
and 2001 (overlay)

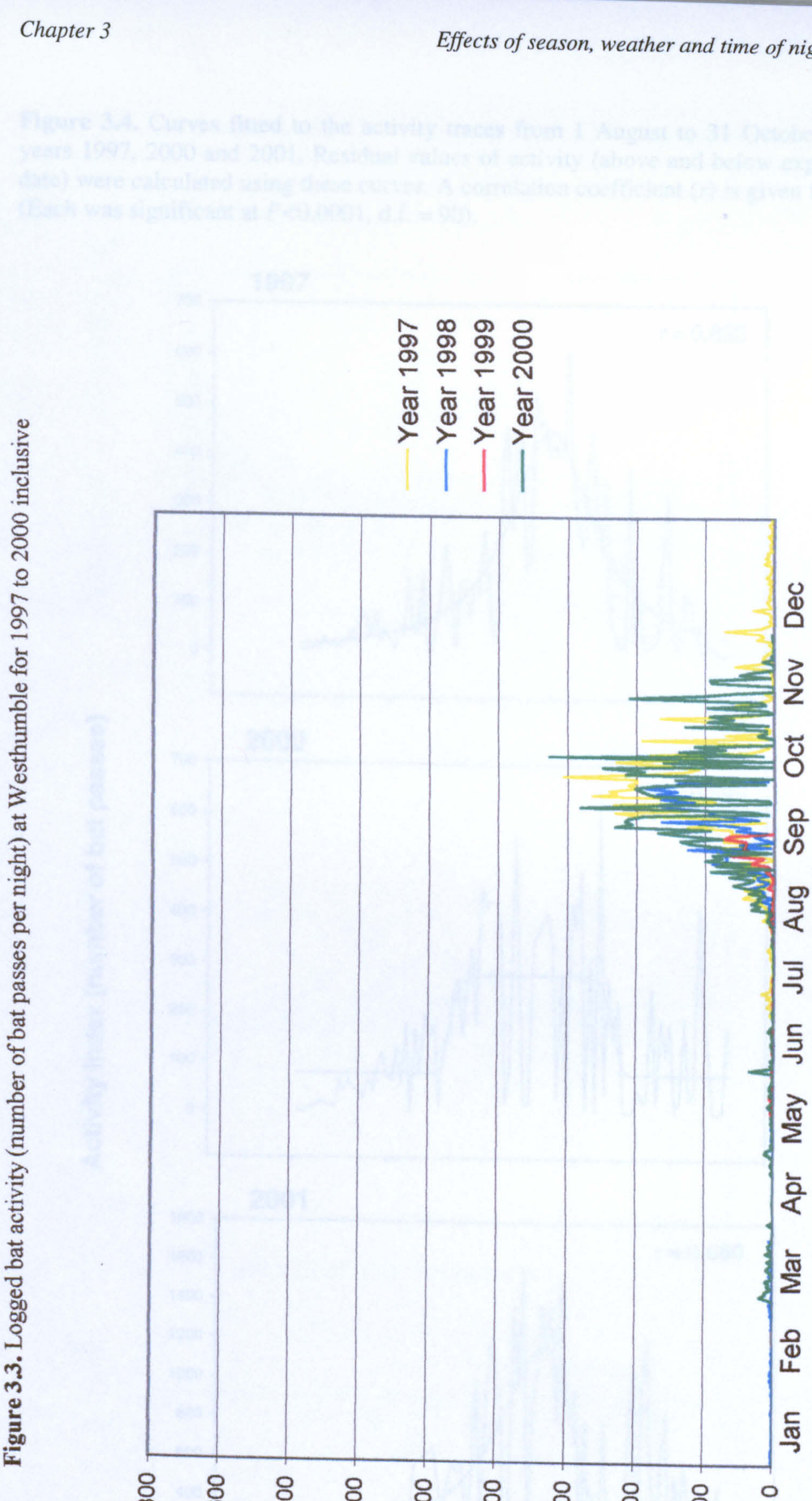


Figure 3.3. Logged bat activity (number of bat passes per night) at Westhumble for 1997 to 2000 inclusive

Figure 3.3. Logged bat activity (number of bat passes per night) at Westhumble for 1997 to 2000 inclusive and 2001 (overlay)

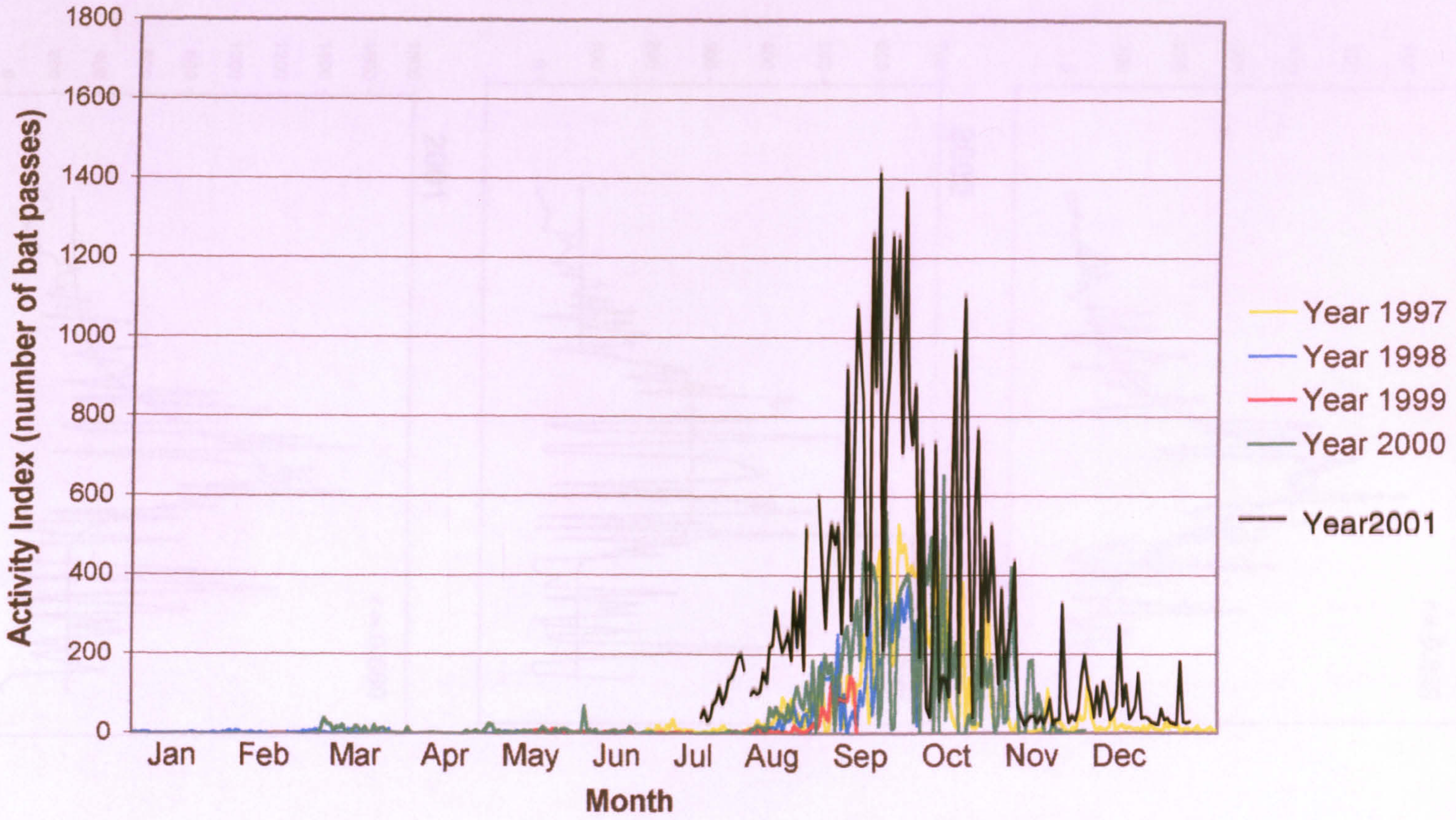


Figure 3.4. Curves fitted to the activity traces from 1 August to 31 October inclusive for years 1997, 2000 and 2001. Residual values of activity (above and below expected for each date) were calculated using these curves. A correlation coefficient (r) is given for each graph. (Each was significant at $P<0.0001$, d.f. = 90).

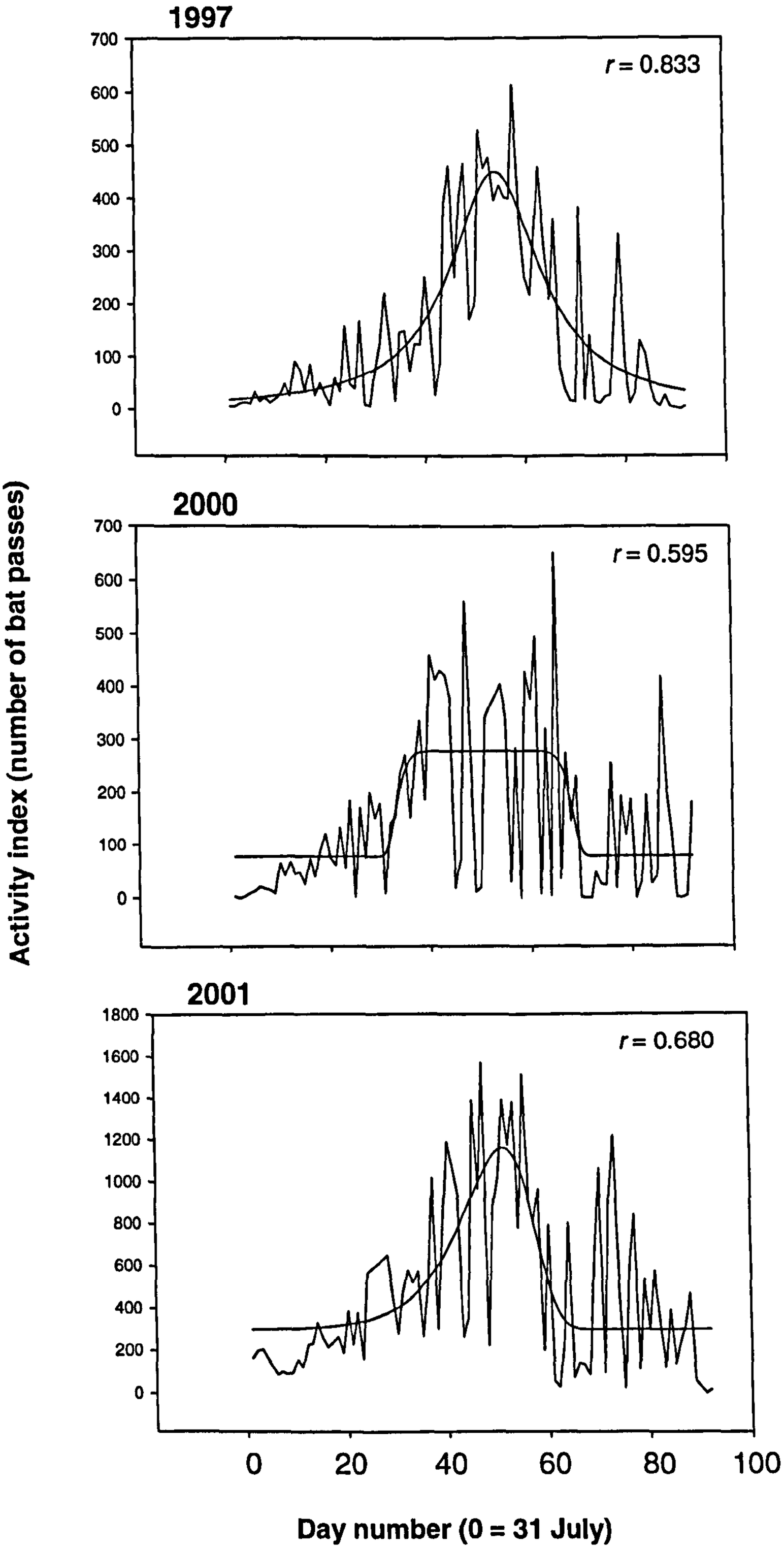


Figure 3.5. **(a)** Residual activity plotted against rainfall. **(b)** Residual activity plotted against residual maximum temperature. The heavy lines show lines of best fit. **(c)** Actual activity index plotted against actual maximum temperature. Data for all graphs is from 1 August to 31 October in 1997, 2000 and 2001.

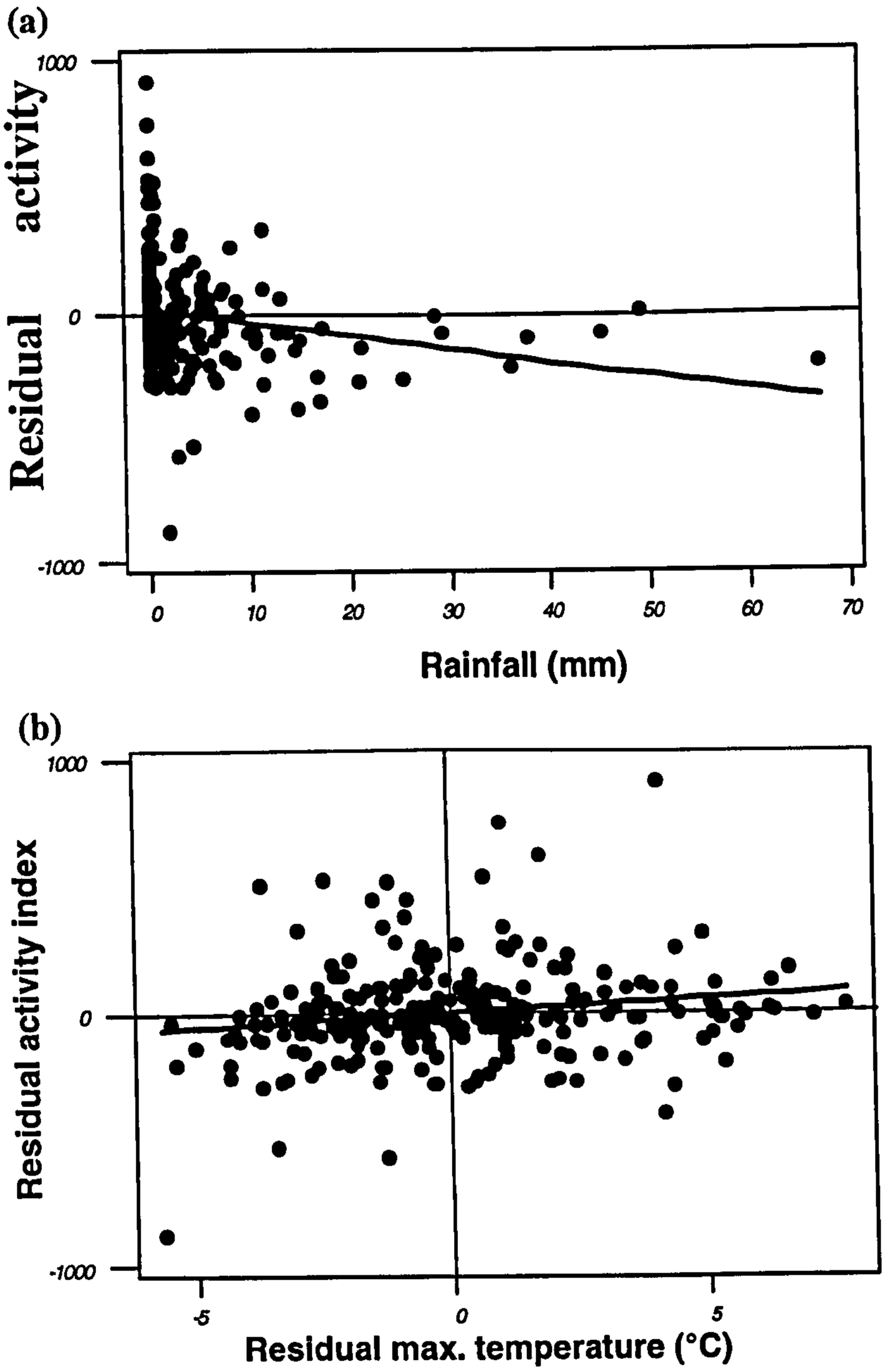


Figure 3.6. **(a)** Residual activity plotted against residual minimum temperature.
(b) Residual activity plotted against percentage of moon face illuminated.
(c) Mean (+ SE) residual activity against moon phase.
Data for all graphs is from 1 August to 31 October in 1997, 2000 and 2001.

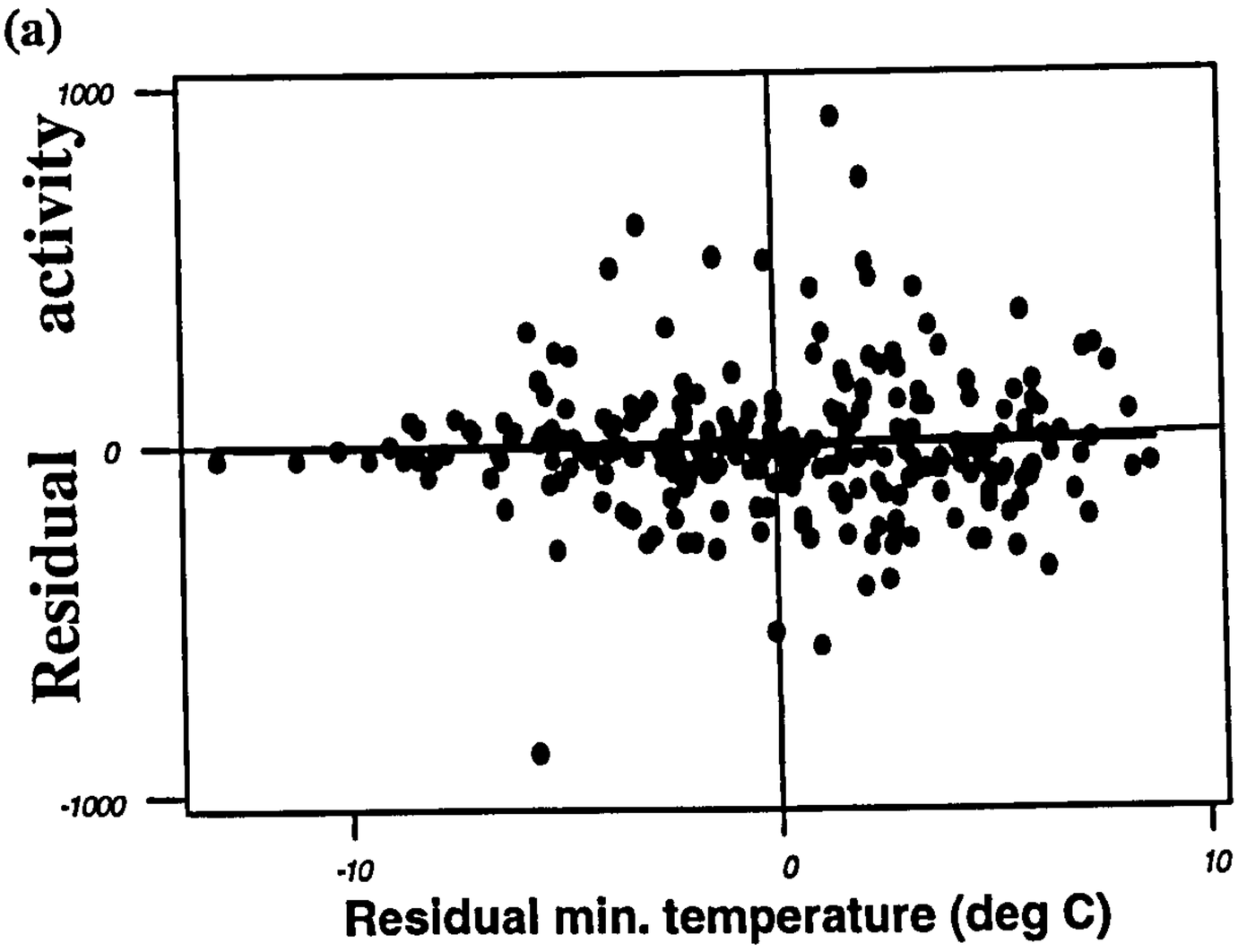


Figure 3.7. Mean hourly activity over a 24-hour day for each half-month during the swarming season at Westhumble. The black bars beneath the *x*-axis represent the hours of darkness.

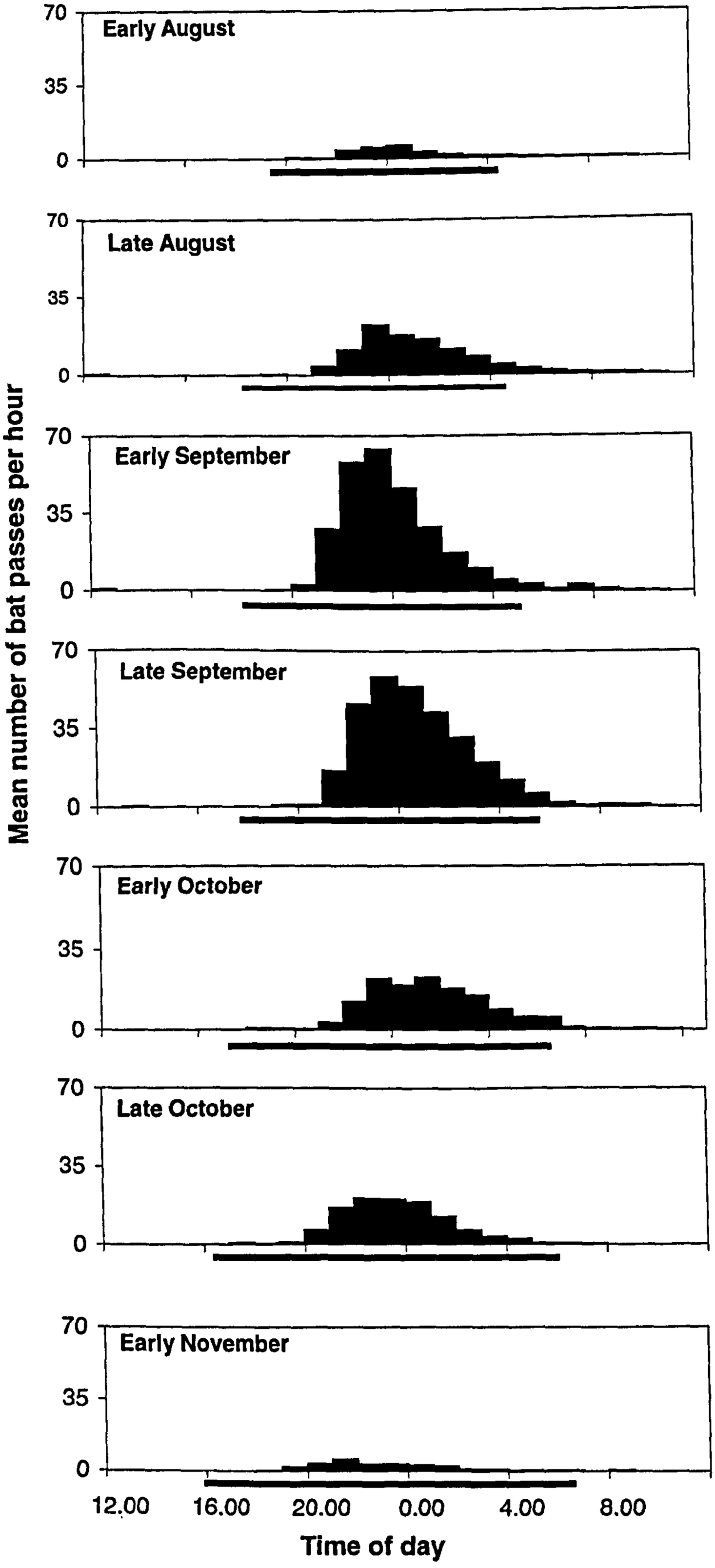


Figure 3.8. Number of vespertilionid bat calls logged per hour after sunset at Byfield on 18 September 2000 at logger locations 1 and 3, 30m and 300m inside the entrance respectively (see Fig. 3.1).

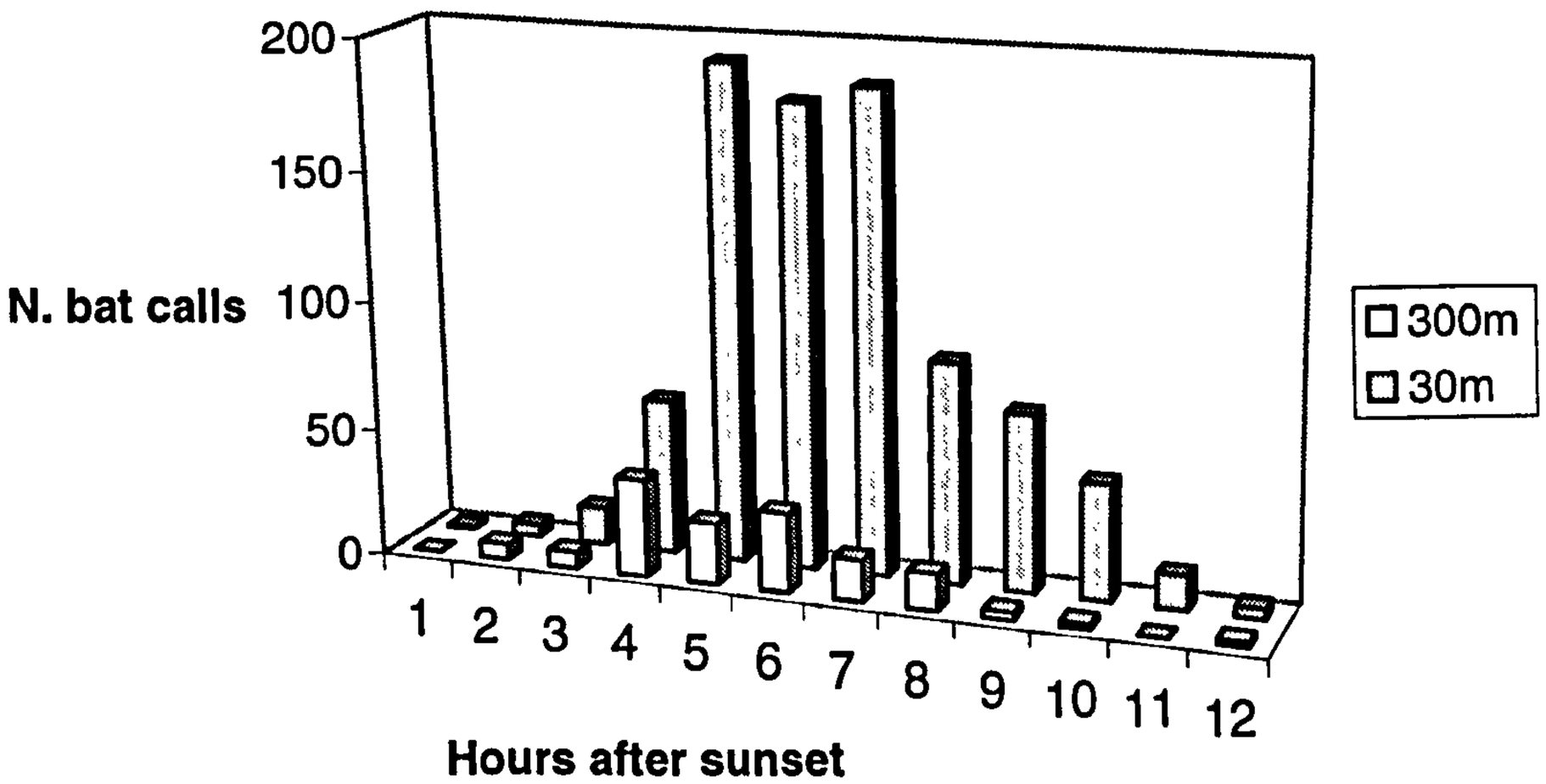


Figure 3.9. Mean (± SE) number of vespertilionid bat calls logged per night at Byfield during the swarming season (July – October) at each logger location (see Fig. 3.1). The number of logging nights represented by the data is given in brackets.

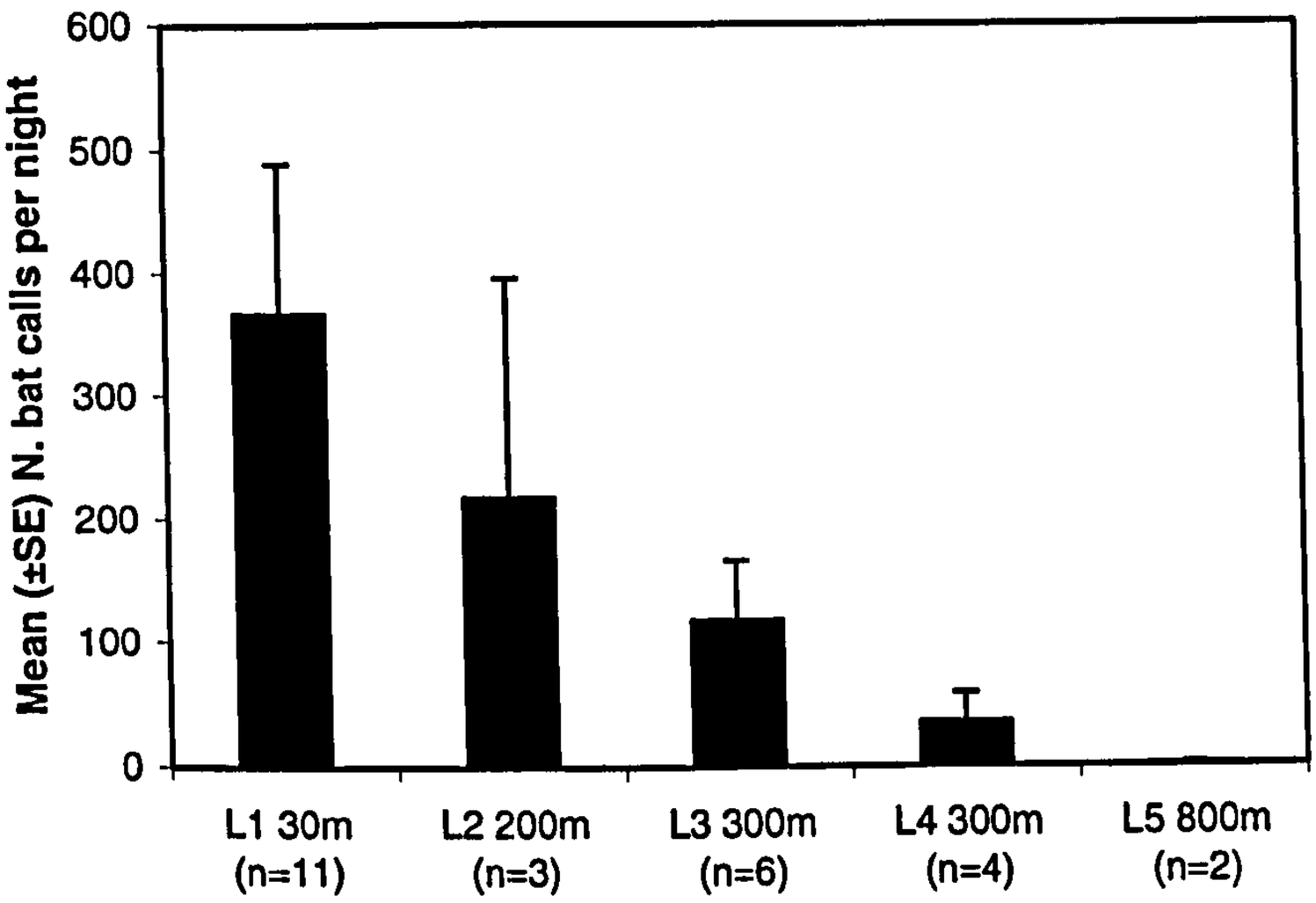
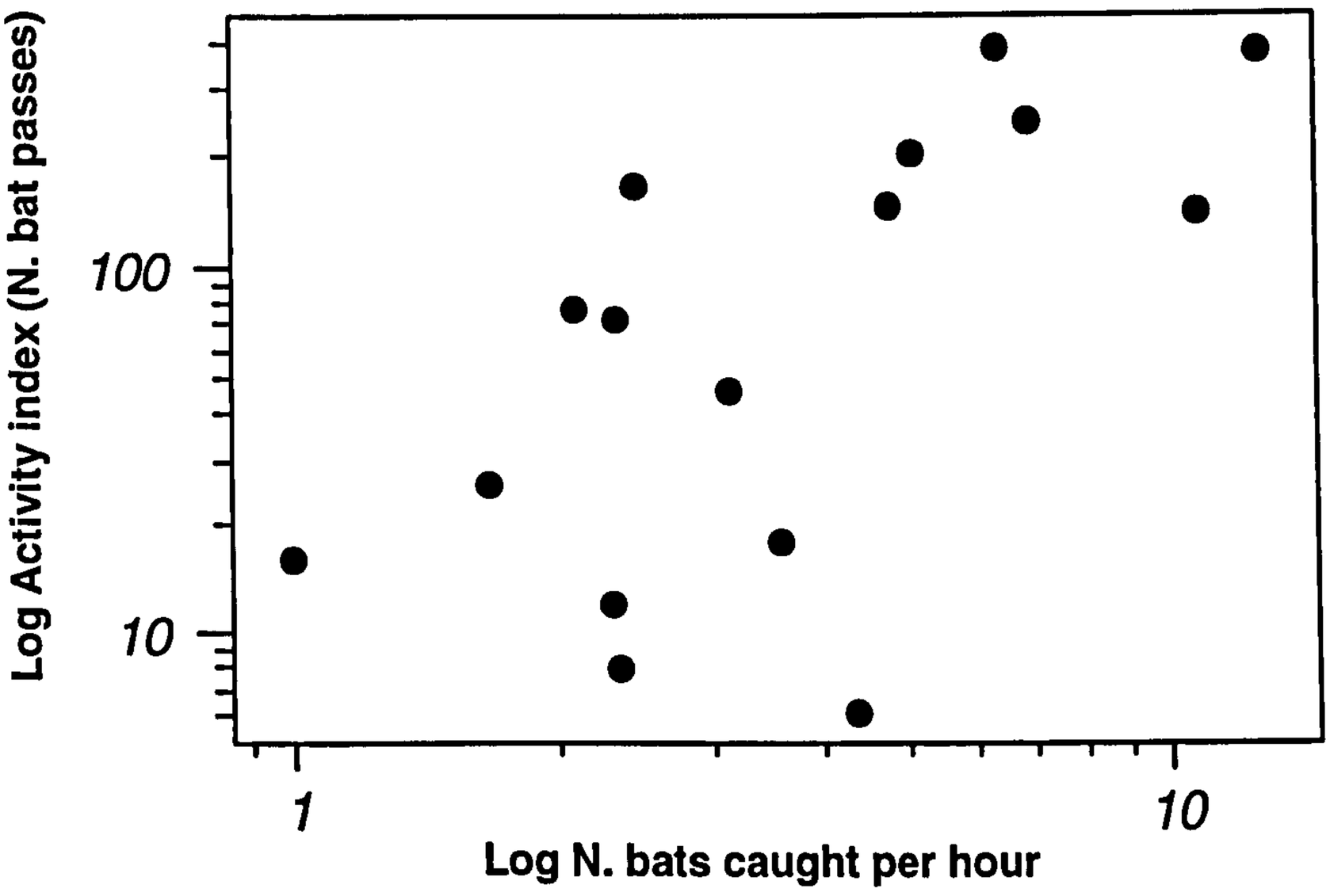


Figure 3.10. Correlation of number of bats caught per hour (log) with nightly activity index (log) recorded by the logger on catching nights.



3.4. DISCUSSION

3.4.1. Annual activity

Activity loggers can clearly delineate the period of swarming between August and October and the continuing lower level of activity attributable to the onset of hibernation during November and December. Discrepancy between the level of activity in 2001 and in all other years in this dataset is most likely due to the equipment having greater sensitivity at the same gain in 2001 after the unit was re-built following flooding. This discrepancy was not discovered until after data collection had been completed. There was no difference in the number of bats caught in 2001 compared to previous years, so a real increase in bat numbers and/or activity during swarming is thought unlikely. Considerable variation in activity was recorded from one night to the next consistent with findings by Humphrey & Cope (1976).

3.4.2. Correlation of activity with environmental variables

The clearest relationship between activity and a climatic predictor was for rainfall. Rainfall, especially heavy rainfall (>15 mm per 24-hour period), suppressed swarming activity. Erickson & West (2002) also found a negative association of bat (foraging) activity with rain. Bauerová & Zima (1988) observed a decrease in the intensity of bat activity in rainy weather. In contrast, Navo *et al.* (2002) reported that activity at a swarming site remained high throughout one night's survey despite a thunderstorm and Berkova *et al.* (2002) reported no significant effect of rain on bat activity at a cave entrance. Perhaps swarming activity is suppressed if rainfall is persistent at the time of emergence and prolonged through the evening. If bats have already traveled to the swarming site before rain commences, and particularly if the rain is of short duration, it may have a reduced effect on activity levels. The influence of wind speed on swarming activity was not investigated here. It should be taken into consideration in future analyses.

After controlling for season, ambient temperature was positively correlated with swarming activity, in agreement with Sendor's (2002) findings for swarming *P. pipistrellus*. Bats are more likely to visit a swarming site when it is warmer than expected. Favourable nights for swarming might be those when temperatures are beneficial for flying insects and therefore also for bat foraging (Williams, 1961). Bats would be able to obtain enough energy to cover costs rapidly before embarking on a potentially long distance journey to a swarming site, energetic flight activity during swarming and a return flight. I assume that the same climatic influences will operate on all species that swarm, however in reality some species might be better adjusted to coping with colder conditions than others. For example, the foraging strategy of *M. nattereri* (gleaning) may explain why it swarms later in the year than *M. daubentonii*, *M. brandtii* and *M. mystacinus* (aerial hawkers) (Chapter 2, Parsons *et al.*,

2003). Differences between the species in response to different temperature conditions could also operate from night to night in addition to seasonally during swarming.

Activity levels did not vary with minimum nightly temperature (likely to be in the early hours of the morning), indicating that the temperature at emergence may be of greater influence to a bat's decision of whether to swarm or not. Sendor (2002) found that mean ambient temperatures described swarming activity better than minimum temperature. He concluded that flight activity of insects at dusk would be more influenced by daily mean temperature than by nightly minimum temperatures and would consequently have more influence on swarming activity for the reasons given above.

I found activity to be suppressed below a maximum temperature of around 13°C. The decrease in temperature responsible for onset of hibernation (Erkert, 1982) might also trigger the decline in swarming behaviour. Due to a maritime climate, hibernation at Westhumble typically begins around 15-20 December (F. Greenaway, pers. comm.). Prior to this bats may have entered torpor in trees but continued to become active on some evenings for foraging.

In agreement with a recent study by Karlsson *et al.* (2002), swarming activity was not significantly affected by moon phase, despite a trend for reduced activity on nights with a lot of illumination by the moon. Degree of cloud cover was not accounted for; therefore the results may have been affected by nights with full moon and heavy cloud cover during which ambient light levels would be much reduced compared to a night with full moon and clear skies. A more accurate measure of ambient light each night would have been to use a light meter such as that used in a photographic dark room (Hecker & Brigham, 1999).

3.4.3. Nightly activity

Although at a higher magnitude during peak swarming in September the pattern of nightly activity was similar throughout the swarming season. The pattern of low activity after dusk and a peak in the middle of the night is consistent with few bats being resident in the mine during the day (Humphrey & Cope, 1976) and many bats arriving from the surrounding areas to swarm for several hours and then departing again prior to daybreak. Radio-tracking has shown that bats can live up to 27 km from a swarming site during the swarming season and are capable of completing the round trip in one or two nights (see Chapter 5).

Degn *et al.* (1995) distinguished two phases during the swarming period, one with equal flights into and out of the mine and activity spread throughout the hours of darkness, and a later phase with dominant flights in during the hours before dawn, explained as the onset of

hibernation. Separate phases were not distinguishable from the nightly data in this study. The distinct period of bats entering the mine may result from extremely cold nights in Denmark, because of the more northerly latitude and continental climate, giving a more marked start time to hibernation than in Britain. The population in Degn *et al.*'s study (1995) primarily comprised *M. daubentonii* whereas in our study more species were present. The peak found by Degn *et al.* (1995) in October representing onset of hibernation of *M. daubentonii* was probably masked in our study by the continued swarming of *M. nattereri.* Sendor *et al.* (2000) also found that activity was more concentrated for *P. pipistrellus* during the pre-hibernation period, but that most activity was immediately after sunset rather than before dawn. Again, this study was conducted in a continental rather than maritime climate, perhaps explaining why no distinction could be made between nightly activity patterns during swarming and during the onset of hibernation.

The pattern of nightly activity at Byfield was the same as at Westhumble. Placement of loggers at different locations within the system at Byfield showed that bat activity decreased with distance into the mine. The majority of activity was concentrated in the entrance region; there appears to be no reason for the bats to penetrate deeply into the mine. The areas in which most activity was found is also the area where most vespertilionid bats hibernated (R. D. Ransome, pers. comm.). Fenton (1969) also found that bats tended to hibernate in the part of the mine in which they were caught, observations which perhaps support the theory that swarming bats are visiting to assess potential hibernation sites, although hibernation counts have not been conducted recently at Byfield for safety reasons.

3.4.4. Logged activity and capture of bats

The number of bats captured per hour was significantly positively correlated with the number of bat passes; therefore the logger is representative of the number of bats present on any one evening. Degn *et al.* (1995) also reported that their logger findings corresponded well with trap captures. There were no differences between levels of activity on catch days compared with the day prior to or following catching indicating that activity is not adversely affected by the catching. This result is at first surprising because capture of bats is considered to have great disturbance impact and bats have been shown to develop trap shyness, particularly from one night to the next (Duffy *et al.*, 2000; Kunz & Anthony, 1977). The apparent absence of a detrimental effect of catching on activity levels in this species may, in part be explained by different cohorts of bats arriving on consecutive evenings (Davis & Hitchcock, 1965; Hall & Brenner, 1968; Harrje, 1994; Whitaker & Rissler, 1992). A novel group of bats would be unaware of the disturbance on the previous evening.

3.4.5. The future of automatic logging

Activity recorded by loggers corresponds well with numbers of bats captured and gives a more accurate representation of the use of an underground site than hibernation counts (Baagøe *et al.*, 1988; Degn *et al.*, 1995), particularly at sites where bats are seldom seen in hibernation because they hide in crevices. Permanent installation of logging systems at swarming sites would permit long-term monitoring of visitation by bats. Population increases or declines would be evident across years, but only if calibration of equipment remained consistent. Comparatively cheap systems would provide a great deal of information and reduce the time, labour and disturbance inherent in catching surveys and hibernation counts. Advances in technology used for recording and analyzing ultrasound should enable accurate and reliable distinction between species with similar echolocation in the future, which could be incorporated into loggers to provide information on the use of a site by different species.

CHAPTER 4

THE SIZE OF SWARMING BAT POPULATIONS

4. THE SIZE OF SWARMING BAT POPULATIONS

SUMMARY

149 *M. bechsteinii*, 737 *M. daubentonii* and 1476 *M. nattereri* were fitted with uniquely numbered rings at swarming sites, at day roosts and in foraging areas. Of these 9% have been recaptured or found at least once. Overall recapture rate was highest for *M. daubentonii* (10.7%) and was similar for *M. bechsteinii* (7.3%) and *M. nattereri* (7.7%).

Fewer females were recaptured than males indicating that males visit a site more often during swarming. At the main study site there were more recaptures between swarming seasons than within one swarming season, indicating that return to the site is low during the same year, but that bats are faithful to the site from year to year. Some bats hibernated at the site at which they swarmed.

The sizes of swarming populations of *M. bechsteinii, M. daubentonii* and *M. nattereri* were estimated by three techniques: (1) a multiple-capture closed population model allowing for variation in capture probability by time and by individual, (2) a basic population estimator based on the Lincoln-Petersen method, and (3) extrapolation from the number of bats caught during swarming.

Depending on trap capture efficiency, population sizes were estimated as between 145 and 850 for *M. bechsteinii*, between 860 and 8300 for *M. daubentonii* and between 3470 and 18100 for *M. nattereri.* Thus there is considerable discrepancy between estimates. I have most confidence in the Lincoln-Petersen and the closed capture estimates because they were of the same order of magnitude, and least confidence in the extrapolation method because it might have been unduly influenced by one or two particularly large capture occasions during fair weather and because capture efficiency of the traps was not known. Thus I conclude that around 150-200 *M. bechsteinii,* 1000 *M. daubentonii* and 3500-4000 *M. nattereri* visit Box stone mine annually during swarming.

92% of recaptured bats showed no evidence of injury caused by the ring. 6% had mild injuries and 2% had injuries classed as severe.

4.1. INTRODUCTION

Marking animals for individual identification opens up many avenues of investigation to the researcher. Re-catching or re-sighting marked animals provides data on dispersal away from or return to the point of release. Such recapture data can be used to estimate population sizes, survival rates and other demographic parameters. In addition, known animals can be studied over time to obtain individual specific information about such things as breeding success, body mass changes and longevity. The method of marking usually employed for bats is ringing (banding) of the forearm with a uniquely numbered metal or plastic ring (Barclay & Bell, 1988). Other methods include fluorescent light-tags (Barclay & Bell, 1988) and passive integrated transponders (PITs) (Kerth & König, 1996).

As discussed in Chapter 2, catches at swarming sites in southern Britain have revealed large numbers of bats of up to a dozen species visiting on a nightly basis. Marking individuals of three of these species allowed me to ask how often the bats return, how big the populations are, whether bats visit other swarming sites, how far they travel and whether they hibernate at the same site at which they swarm. Marking bats at swarming sites has the potential to contribute information to and thereby improve the accuracy of nation-wide population estimates, which at present are acknowledged to be inaccurate for many British species (Macdonald & Tattersall, 2001), and to further increase our understanding of swarming behaviour.

4.1.1. Rate of return to swarming sites

Recapture of ringed bats is generally low during swarming (Davis & Hitchcock, 1965; Fenton, 1969; Hall & Brenner, 1968; Harrje, 1994; Humphrey & Cope, 1976; Whitaker & Rissler, 1992) suggesting that turnover is high and the population is correspondingly large and may occupy an extensive catchment area (see Chapter 5). At sites in North America hundreds to tens of thousands of bats have been ringed to study seasonal movements and fidelity to hibernacula. For example Davis (1964) ringed more than 12,000 bats at one cave in Kentucky in 17 days during autumnal swarming and discovered that *M. sodalis* can make a round trip of over 600 miles within nine days. In one of the largest studies of its kind Davis and Hitchcock (1965) ringed over 73,000 bats at caves and in summer colonies in northeastern USA and southeastern Canada. Despite this huge number no bat ringed during the swarming season at the best-studied cave was ever recaptured there in the same season, even when the population was examined the following day and night (Davis & Hitchcock, 1965). Fidelity to the site was suggested because only on two occasions were bats found at another cave, one later in the same year and another a year later (Davis & Hitchcock, 1965). Similarly, Humphrey and Cope (1976) recaptured few *M. lucifugus* during the same

swarming season and there was little movement to other caves. Whether bats have been found at other caves since the publication of these papers is not known. Recapture rate between years at Renfrew mine varied from 1.5% to 4.3% (Fenton, 1969). More adult males (95%) returned than adult females, but return was more even between the sexes for juveniles (46% male) (Fenton, 1969). I predict that recapture rate of bats at a swarming site will in general be low, but may be higher for males than for females. Recapture may be higher between years than within the same swarming season (particularly for females) and because of the high turnover, I predict that populations visiting such sites will be very large.

4.1.2. Estimating populations

A population is a group of organisms of the same species occurring in a particular space at a particular time (Krebs, 1994). For the purpose of this study 'population' refers to the population present at the swarming site during the swarming season. At any one time the population at the swarming site is a sample of the entire population within the catchment area.

Statistics can be used on recapture data to provide estimates of population size (with error limits) for elusive, mobile organisms (such as bats) that are seldom if ever amenable to direct counts (Montgomery, 1987). However their accuracy usually depends on capturing a large proportion of the population (Southwood & Henderson, 2000). Although many researchers have marked bats for later identification, only twice have researchers attempted to estimate the number of bats visiting a swarming site by using mark-recapture statistics. Sendor (2002) used closed models (Otis *et al.*, 1978) to estimate the size of populations of adult males and females and juveniles of swarming *P. pipistrellus* at a castle in Germany. Bauerová & Zima (1988) estimated the size of the swarming bat community by pooling all species and using the Lincoln-Petersen estimator for each year of study. I consider it erroneous to pool all species because they may be heterogeneous in their capture probability due to differences in ecology. In addition, the Lincoln-Petersen index is the simplest measure of populations and can only be used on 'closed' populations, ones in which there are no births, deaths or migrations. A minimum of three years of recapture has been suggested for a viable study in bats (Stebbings, 1988). My study was conducted between 1999 and 2002, therefore the swarming populations are considered to be 'open' (subject to births, deaths and migrations) over the entire period of study. However a situation may be considered where the population is regarded as closed (with no births and negligible mortality) within a swarming season but open between seasons, hence closed models can be used on data from each season, but not between seasons. Regardless of whether a population is open or closed, several assumptions of capture-mark-recapture models must be met (Southwood & Henderson, 2000).

Assumption 1. Animals are unharmed and unaffected by their marks after release, and they are recognizable again on recapture (Southwood & Henderson, 2000). Ringing of bats with one forearm ring has been an accepted method of marking bats for many years and it enables easy and reliable recognition of individuals. However, during the period of my study the safety of ringing bats was questioned by a team of Australian researchers (Baker *et al.*, 2001), prompting some discussion of the topic (Jones, 2002a). Baker *et al.* (2001) found unacceptable levels of injury from certain designs of ring, including those intended for use on birds and old-type bat rings. The level of injury caused by the newer type of rings (the type used in my study) was found to be minimal (0.07%) by Colin O'Donnell (Baker *et al.*, 2001) and by Entwistle *et al.* (2000). Roger Ransome has recorded no injuries caused by rings on *R. ferrumequnium* during a 15-year period (Jones, 2002a). Therefore, if correctly applied, bat rings are assumed not to affect the longevity or the behaviour of the animals. Through activity logging (Chapter 3) I showed that activity at a site was not adversely affected by capture of bats.

By double-ringing Entwistle *et al.* (2000) estimated ring loss at less than 3%. I did not use double-ringing with two rings on one forearm for fear that it would decrease the foraging success and correspondingly the survival rate of the bats. Rings knocking against one another produce ultrasound, which might alert tympanic moths to the bat's presence (Norman *et al.*, 1999). I did not use double-ringing with one ring on each forearm because the ability to determine sex of the bat if seen in hibernation (by which ring the arm is on) would be lost. For the purpose of this study the application of rings is assumed to be permanent.

Assumption 2. Marked animals completely mix in the population after release (Southwood & Henderson, 2000). There is no reason to believe that marked bats would alter their behaviour away from the study site, or disperse in a different manner to unmarked animals. Due to their mobility marked bats would mix into the population rapidly after release (as shown by the radio-tagged bats which were also ringed – see Chapter 5).

Assumption 3. All bats are equally catchable (Southwood & Henderson, 2000). Age groups and sexes should be sampled in the proportions in which they occur. It has been suggested that juvenile bats may be more likely to be caught than adults due to reduced experience (Kunz & Anthony, 1977; Trappmann, 1997). This may be true during the first few flights from a maternity roost (Kunz & Anthony, 1977). However, by the time my study was conducted juveniles had been volant for several months, and for most bats (adults and juveniles) the trap(s) would be a novel experience, therefore different age groups are assumed to be equal in catchability. Trappmann (1997) explained the male bias seen in catches during

swarming as a capture bias, perhaps because males were more frenzied in their flight activity than females, thus making them more susceptible to capture. However, there is no further evidence to support this and consequently males and females are assumed to be equally catchable.

Due to the length of time between sampling occasions I assume that trap shyness is negligible, unlike in studies where catching was carried out on consecutive nights (Degn, 1987b; Duffy *et al.*, 2000; Hall & Brenner, 1968; Kunz & Anthony, 1977). During swarming catches I have sometimes captured one individual up to four times on the same night indicating that, either that they do not learn the location of the trap, or that they are extremely persistent in their attempts to enter the mine and are not shy of the trap placed in their path. It has been suggested that traps should be placed randomly, or several trapping methods be used to minimize trap shyness but this was not possible and would violate Assumption 5 that catching effort is consistent. The traps were designed and placed to maximize the number of captures and moving them or using different methods were not feasible.

Probability of capture will depend on the seasonal patterns of behaviour shown by the species. For example for four months of the year no *M. nattereri* may visit the swarming site because they are resident in day roosts and foraging areas in the surrounding countryside, for the next four months they may be found in great numbers on some nights but in small numbers on others due to the nightly variation in activity during swarming, shown in part to covary with temperature and rainfall (Chapter 3), and because of the differential timing of arrival of the species and seasonal nature of swarming itself (Chapter 2). Finally, for the remaining four months of the year they may be present but relatively inactive because they are hibernating. They will arouse infrequently and will not engage in much flight activity, unlike during swarming. Therefore analysis is restricted to catches made during the swarming months when capture probability is more equal, however heterogeneity in individual capture probability and variation in capture probability due to time can be accounted for in some statistical models.

Assumption 4. Sampling takes place at discrete time intervals and the length of time spent sampling is small in relation to the total time of the study (Southwood & Henderson, 2000). This assumption is met by sampling only once per fortnight during the swarming season (roughly three months duration) in each year.

Assumption 5. Catching effort is the same on each occasion. The location and number of traps used was kept consistent at each of the sites. The hours of trapping varied according to

the length of the night, weather conditions and the researcher, hence is not consistent across all sites. Trapping duration was most consistent at Box, where most of the ringing was carried out, and it is for this site that population estimates will be calculated.

The models

There are various closed and open population models and combinations of both that could be applied; however deciding which model should best be used is difficult, particularly because many of the recent models focus on estimating survival and other parameters of open populations before estimating the size of populations (Lebreton *et al*, 1992).

Ideally an open population model would be applied to data from all capture occasions combined. The Jolly-Seber model is most frequently used for open populations (Kendall *et al.*, 1995), but is not appropriate here because it requires a high proportion of recaptures and can lead to biased estimates unless each sample contains a large proportion of the population (>50%) (Greenwood, 1996) which is unlikely.

An important development applicable to animals studied for an intensive period every year for several years is the robust design (Greenwood, 1996; Pollock, 1982). It is a flexible *ad hoc* approach that combines features of open and closed population studies (Southwood & Henderson, 2000). It is assumed that there are gains and losses (births in the summer and deaths over winter perhaps) between k primary periods, but during each primary period of study there are l secondary samples during which gains and losses are assumed to be negligible (perhaps during a swarming season). A closed population model is applied to data from the secondary samples to estimate population size for each primary period, which decreases biases from unequal catchability (Kendall *et al.*, 1995). Open models, such as Jolly-Seber, are applied to pooled information from the primary periods to estimate survival. The period during which secondary samples are taken should be short relative to the duration between primary periods and there should be a minimum of five secondary samples per primary period (Montgomery, 1987). This robust design gives better estimates of population size and turnover rates than the Jolly-Seber method alone (Greenwood, 1996) and has been recommended for small mammal studies by Nichols *et al.* (1984) and Nichols and Pollock (1990). However, unless sufficient data are gathered this method may not be appropriate due to large standard errors.

If each swarming season is treated independently then multiple-capture closed models such as those of Otis *et al.* (1978) might be appropriate in an approach based on the robust design. Closed capture models can be adjusted to account for variation in capture probability with

time, behaviour and individual (heterogeneity). A model incorporating variation in capture probability according to time and individual heterogeneity (M_{th}) and the simple Lincoln-Petersen estimator used by Bauerová and Zima (1988) will be applied here.

Extrapolation based on activity

In addition to the statistical methods of population estimation I will attempt to produce an estimate based on the numbers of bats known to visit on nights during the swarming season. This effectively uses number of bats captured as an index of population size, but it is unlikely that the number of bats captured will correlate with the actual population size. Therefore I cannot use the information in the way for example, number of scats or counts of birds on a lake might be used. This is because, as shown in Chapter 3, bat activity varies greatly from night to night during swarming meaning not all bats will be equally available on any one night. In addition only a sample of those visiting will be obtained because the capture method is not 100% efficient.

By incorporating the amount of variation in the number of bats captured between capture events, the rate of re-visitation and capture efficiency I intend to produce an estimate of the number of bats visiting during the entire season for each species. Baagøe *et al.* (1988) used a similar though not identical method to estimate the number of hibernating *M. daubentonii* from catches during arousals from hibernation. They smoothed the catch data by eye and summed the number expected to have been caught for the remaining nights to produce an estimate of the wintering population of between 3500 and 5000 individuals.

Capture efficiency will depend on whether the bats can detect the traps, how well they can avoid them and the location of a particular trap (for example whether there is space around it or not). Australian researchers found that only 2.6% of approaches to a harp trap in a forested area resulted in capture of a bat (Dobson *et al.*, 2001), although 3.9% of approaches to the harp trap at the main study site in this study resulted in capture (N. Berry & W. O'Conner, pers. comm.). Perhaps this latter figure is slightly higher because the bats were more constrained by the walls of the mine tunnel and hence more likely to fly into the trap rather than around it (Kunz & Kurta, 1988). Because individual bats might have approached the trap on numerous occasions it is impossible to know, without having each bat individually identifiable (perhaps by light-tagging with different coloured light-tags), the percentage of individuals captured from those individuals present. In a situation like at Box, where it is difficult for the bats to circumvent the trap because of the narrow passages and blanking material around the trap, we might assume that every bat present eventually is captured regardless of how many approaches it makes (100% capture efficiency). However there were

occasions when bats bounced off or passed through the lines of the harp trap (author pers. observ., Dobson *et al.*, 2001) and many turned away before attempting to pass. Thus I assume that 100% capture efficiency is unrealistic.

Kunz and Anthony (1977) studied the efficiency of a harp trap outside roosts and found that capture rate varied from 30% to 80% depending on the type of roost exit, colony size and features of age, sex, reproductive conditions and learned responses. Hall and Brenner (1968) claimed 75% capture efficiency for mist-nets at a swarming site and Degn (1987) claimed 90% efficiency of the harp trap in his study, yet it was positioned such that fewer echoes reflected back to the bats than from a conventional harp trap. In the absence of knowledge of actual capture efficiency I will extrapolate populations at several different capture efficiencies.

4.1.3. Study species

M. daubentonii and *M. nattereri* were selected because they were most common at the site (Chapter 2), so a large sample size could be obtained, and because they were also the subject of the radio-tracking project to determine the catchment area of the swarming site (Chapter 5). In addition *M. bechsteinii* were fitted with rings. Although less abundant than the other two species, a population estimate will be of great interest because the current population size is unknown and breeding colonies of this species have only recently been discovered in Britain (Macdonald & Tattersall, 2001).

The specific aims of this chapter are:

1. To discover the rate of return of bats to a swarming site and to determine whether bats hibernate at the site at which they swarm.
2. To estimate the size of swarming populations of *M. bechsteinii*, *M. daubentonii* and *M. nattereri.*
3. To assess whether injuries are caused to the bats by the rings.

Movement of ringed bats to other swarming sites and elsewhere in the study area will be addressed in Chapter 5.

4.2. METHODS

4.2.1. Study sites

Six major underground study sites were the focus of the mark-recapture programme, which commenced in August 1999 (Fig. 4.1. See Chapter 2 for site descriptions). Bats were caught as described in Chapter 2. Bats were ringed at an additional 10 locations (2 day roosts, 2 mine entrances and at 6 locations adjacent to or over waterways) throughout the study area in 2001 and 2002 (Fig. 4.1). At these sites, bats were captured by mist net or harp trap, except at Forest Farm where a hand-net was used to catch bats on emergence from their day roost. These sites were chosen because they were known or suspected *M. nattereri* roosts, because they had been found through radio-tracking from the study site (Chapter 5) or because they were suspected foraging areas of *M. daubentonii*.

4.2.2. Ringing procedure

Myotis bechsteinii, M. daubentonii and *M. nattereri* were fitted with 2.9 mm aluminium bat rings (Model 1BR3521, The Mammal Society, London supplied by Robert Stebbings Consultancy, Peterborough, UK and manufactured by Lambournes Ltd.) (Plate 4.1). Males were ringed on the right forearm and females on the left to enable sex determination if the animal could be viewed in hibernation but not reached to read the ring number. Most bats captured on each night were ringed, although a very small number were not, either because we ran out of rings or because the bat escaped before ringing. This number is so small as to be assumed to be unimportant to the analyses. Ringing was carried out under English Nature license by the author, Gareth Jones, Ian Davidson-Watts and Steve Laurence.

4.2.3. Recapture procedure

When bats were recaptured or seen in hibernation[1] the ring number was recorded and any injury was noted. Injuries were regarded as 'severe' (puncture of wing membrane by the ring or skin growth preventing movement of the ring) or 'mild' (small holes in the wing membrane, irritation or swelling of the skin of the forearm, but ring still able to move). Ring damage was noted when there was evidence of teeth marks or the shape of the ring had changed. Where recaptures had suffered severe ring injury the ring was removed and replaced on the other arm. When ringed bats were seen during hibernation counts (see Chapter 2 for methodology) the ring number was read and recorded if possible.

[1]Hibernation counts were coordinated by I. F. Davidson-Watts and S. Laurence.

4.2.4. Data analysis

I decided to analyse each species and sex separately, firstly because there may be ecological differences between species and the populations should be considered independently, and secondly, because ecological differences between males and females might influence their capture probabilities. However because very few females were either ringed or recaptured analyses could be performed for males only.

Multiple-capture closed method

Data were entered into the program MARK (Version 3.0, White & Burnham, 1999) as encounter history matrices for male *M. daubentonii* and male *M. nattereri* for each swarming season. Data were insufficient for male *M. bechsteinii*. No distinction was made between juveniles and adults as capture probabilities were assumed to be equal. Each set of encounter histories was initially run through the default Closed Capture model in MARK and subsequently through CAPTURE (in MARK) using the model M_{th} of Chao *et al.* (1992). For each year a population estimate was obtained with 95% confidence limits.

Lincoln-Petersen method

In addition, I followed the Lincoln-Petersen method as used by Bauerová and Zima (1988), but I maintained separation between the data for each species. Where sample sizes are small, population sizes may be over-estimated by the most basic Petersen formula (Krebs, 1989); therefore the estimator recommended by Seber (1982, cited in Krebs, 1989) was used. The data for all capture events during the swarming season in each year were pooled and the following equation applied per year of recaptures:

$$N(x) = \frac{(M+1)\,.\,(S+1)}{R+1}_{-1}$$

Where N is the estimated size of the population in year x
S is the total number of individuals captured within year x
M is the number of individuals banded in the year(s) preceding year x
R is the number of individuals banded previously and recovered in year x.

Confidence intervals for the resulting population estimates were calculated according to Krebs (1989).

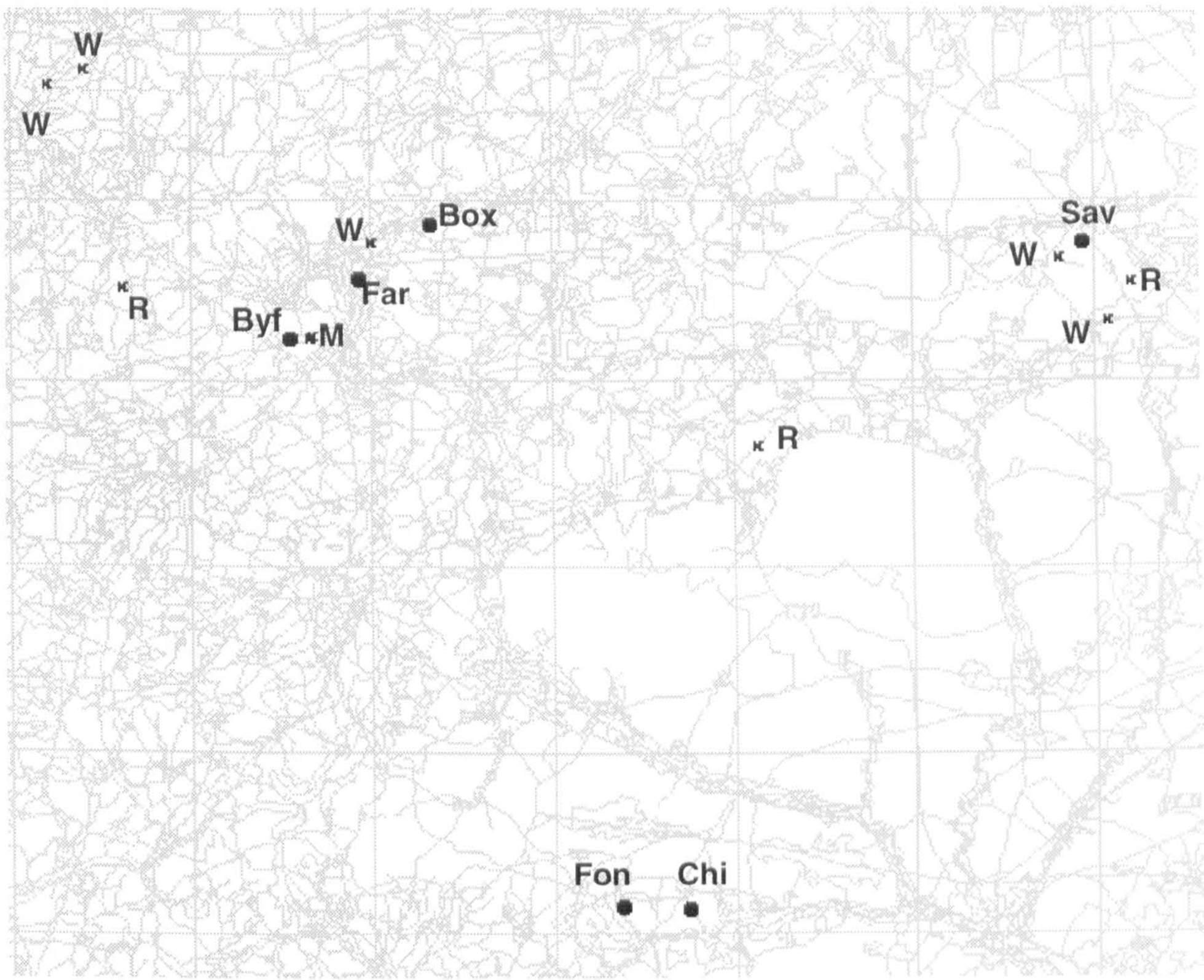
Extrapolation from number of bats caught

Because of the difficulties in fulfilling assumptions of the mark-recapture models I have also attempted to extrapolate the total number of bats of each species using the site during each swarming season from the number of bats captured during capture events.

Firstly, an expected number of males caught per night had catching continued throughout the hours of darkness was calculated based on the distribution of captures hourly through the night for males of each species. Secondly, curves were fitted to these data (expected number of males caught against Julian day) using the same method as for the activity data in Chapter 3. The curve with the highest value of *r* generated from the 'peak' curve fitting function in SigmaPlot (version 5.0) was used to give an expected number of male bats caught had catching been carried out every night during swarming for 90 days (assumed to be the length of the swarming season for each species). Thirdly, different capture probabilities were applied to provide minimum and maximum estimates of the population present during one swarming season. Fourthly, the values were adjusted down according to rate of recaptured individuals (all males) to provide an estimate appropriate to novel male bats only.

For each method of population estimation the estimate for males was adjusted to include females based on the sex ratios calculated for each species at the site during swarming to provide an estimate for the entire swarming population of each species (Table 2.2, Chapter 2).

Figure 4.1. Map showing the locations of the six major underground sites[1] where bats were ringed and additional locations of ringing[2] at roosts, mines and over water. Each square measures 10 x 10 km. The base map is a 1:10,000 Strategi OS map from Digimap (© Ordnance Survey, EDINA/JISC) showing the location of roads and rivers (grey).



[1] Main underground location key

Box = Box stone-mine

Byf = Byfield stone-mine
Chi = Chimark stone-mine
Far = Farleigh stone-mine
Fon = Fonthill stone-mine
Sav = Savernake disused railway tunnel

[2] Additional location key

R = maternity roost of *M. nattereri*
M = mine
W = over water

Plate 4.1. *M. nattereri* with a 2.9 mm aluminium bat ring in place on the left forearm.

4.3. RESULTS

A total of 2362 bats have been ringed since the study began in 1999, comprising 149 *M. bechsteinii*, 737 *M. daubentonii* and 1476 *M. nattereri* (Table 4.1). 2231 (94%) were ringed at the six main study sites and 131 (6%) at the additional sites (Table 4.1). In all species more males were fitted with rings than females because they were more abundant during swarming (Table 4.1).

4.3.1. Rate of recapture of ringed bats

204 individual bats (9% of total) have been recaptured or found at the point of initial capture or elsewhere at least once during the study (Table 4.2). Where bats were captured or found elsewhere it was as a result of catching at other sites by me or other bat workers, or finds by members of the public. Two *M. nattereri* were found dead at Box, presumed killed by a cat or other predator and one *M. nattereri* died (cause unknown) on the second occasion of being caught there. Three additional *M. nattereri* were found elsewhere, also presumably killed by cats. (Note: for map of locations where bats were found see Chapter 5, Fig. 5.3).

Table 4.2. Number of bats of each species recaptured at the site where they were ringed and the number recaptured or found elsewhere. Recapture rate (n. recaptured/n. ringed) is given as a percentage in brackets.

Species	Recaptured at original site	Recaptured away from original site	Total
M. bechsteinii	10 (6.7%)	1 (0.7%)	11 (7.3%)
M. daubentonii	78 (10.6%)	1 (0.1%)	79 (10.7%)
M. nattereri	102 (6.9%)	6 (0.4%)	114 (7.7%)
	3 deceased (0.2%)	3 deceased (0.2%)	

23 bats, all males, have been caught three times and two have been caught four times during the period of study. Proportion of ringed females recaptured was less than the proportion of males recaptured in all three species (Table 4.3).

Table 4.3. Number of males and females of each species recaptured and recapture rate (n. recaptured/n.ringed) given as a percentage in brackets (pooled for all sites).

Species	N. recaptured Males	N. recaptured Females	Total
M. bechsteinii	11 (8.5%)	0 (0.7%)	11 (7.3%)
M. daubentonii	72 (12.4%)	7 (4.4%)	79 (10.7%)
M. nattereri	94 (9.6%)	20 (4.0%)	114 (7.7%)

Table 4.1. Number of bats of each sex of each species ringed at each location, the year that ringing commenced and the number of catching events at each site. The type of additional location[1] (see Fig. 4.1) is given after its name.

Location	Ringing commenced	N. catches	*M. bechsteinii*			*M. daubentonii*			*M. nattereri*		
			Male	Female	Total	Male	Female	Total	Male	Female	Total
Box	1999	35	49	8	57	340	99	439	604	261	865
Byfield	2000	14	5	0	5	45	12	57	64	13	77
Chilmark	2000	6	51	8	59	58	10	68	67	42	109
Farleigh	2000	9	6	0	6	65	7	72	72	24	96
Fonthill	2000	6	17	3	20	41	6	47	62	13	75
Savernake	2001	14	1	1	2	11	7	18	93	66	159
SUBTOTAL		**84**	**129**	**20**	**149**	**560**	**141**	**701**	**962**	**419**	**1381**
By Brook (W)	2002	1				1		1			
Elm Farm (R)	2002	2							2	26	28
Forest Farm (R)	2002	1								39	39
Grey Gables (M)	2001	1							3		3
Kennett (W)	2001	5				5	6	11			
Lincombe (W)	2002	1				1		1			
Oldbury (W)	2002	1				2	8	10			
Marlborough College (W)	2002	3				8	2	10			
Savernake Forest (R)	2001	12							13	12	25
St. Winifreds (M)	2001	1				2	1	3			
SUBTOTAL		**28**	**0**	**0**	**0**	**19**	**17**	**36**	**18**	**77**	**95**
TOTAL		**112**	**129**	**20**	**149**	**579**	**158**	**737**	**980**	**496**	**1476**

[1] R = maternity roost of *M. nattereri*, M = mine, W = over water

GRAND TOTAL 2362

At Box where ringing began earliest and most bats were ringed there were three to four times as many recaptures between subsequent swarming seasons as there were within each swarming season (Fig. 4.2). A very small proportion of those bats caught swarming in any one year were subsequently re-caught that same season (e.g. *M. daubentonii* 1999 = 0%, 2000 = 4.1%, 2001 = 0%, 2002 = 1.4%; *M. nattereri* 1999 = 2.13%, 2000 = 0.81%, 2001 = 2.63%, 2002 = 1.5%). This trend was consistent for both sexes. In both *M. nattereri* and *M. daubentonii* at Box, three females were caught between swarming seasons and one was caught within the same swarming season. Therefore, recaptures of females occurred more frequently but not exclusively between seasons.

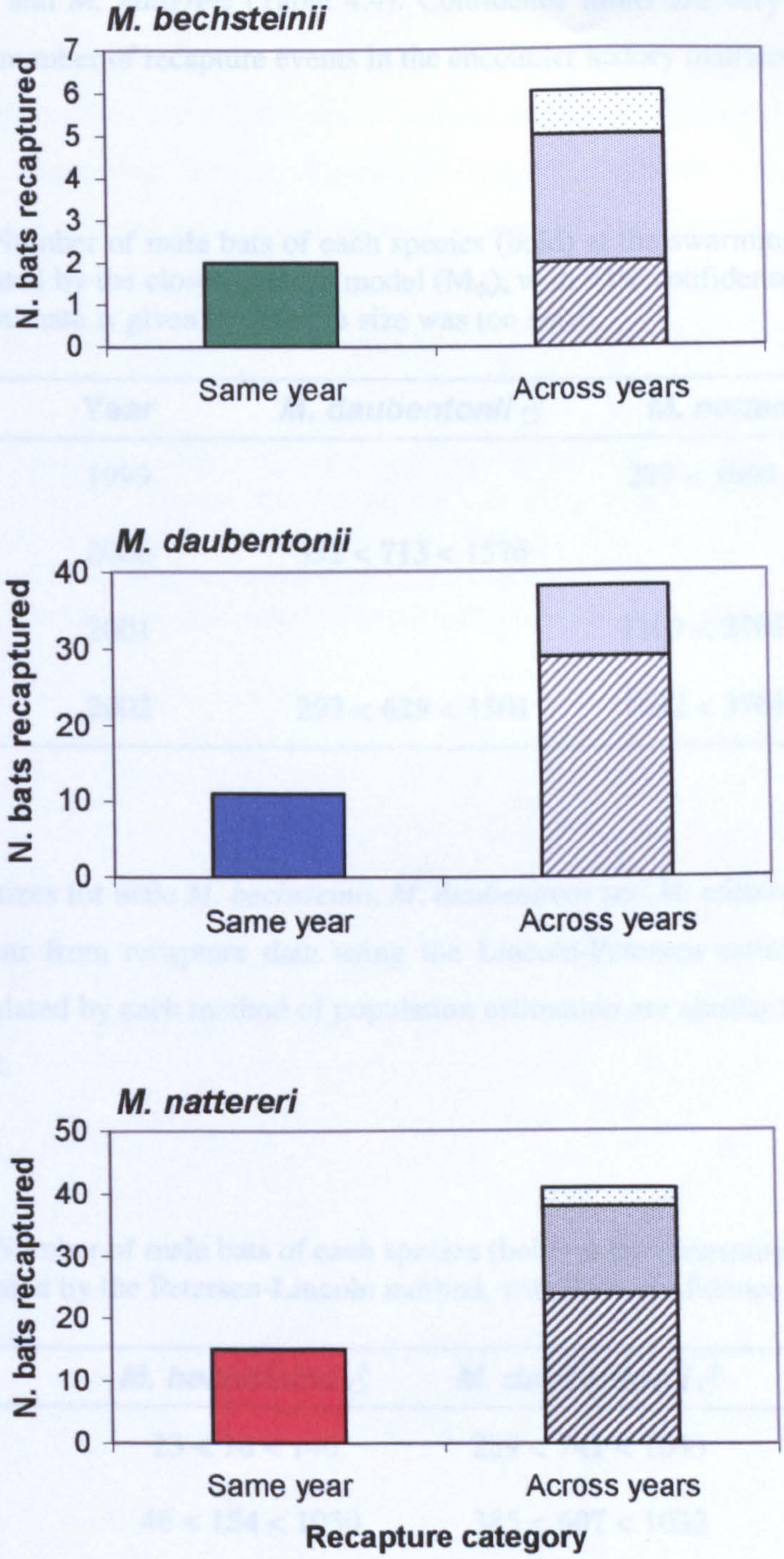
4.3.2. Sightings of ringed bats during hibernation

Three ringed bats (2 male *M. nattereri* and 1 male *M. daubentonii*) have been seen hibernating at Box in the winter after they were captured during swarming there. Most ringed bats have been re-sighted during hibernation at Savernake Tunnel. 14 individuals were observed hibernating in the tunnel in winter 2001/2002 after they were captured there during swarming in the preceding autumn. Five *M. daubentonii* and 31 *M. nattereri* were seen in hibernating in the tunnel in winter 2002/2003 (a further eight ringed *M. nattereri* were seen but could not be reached to read the ring so these are disregarded).

Ringed bats were seen during all three hibernation counts each year (December, January and February) in near equal proportions. Most individuals were only seen during one of the hibernation counts meaning that they must also use other sites, be out of sight, or move above ground at times during winter. However, some were seen on more than one count: two male *M. nattereri* were seen twice in winter 2001/2002; one female *M. daubentonii* was seen in both January and February 2003; two male *M. nattereri* were seen twice; and one male *M. nattereri* was found on all three occasions in winter 2002/2003.

Despite the large number of ringed bats seen in hibernation at Savernake, all of those for which the ring number could be checked had originally been ringed at Savernake. With the exception of the first ringed bat sighted at Savernake, which was believed (though not confirmed) to have come from Box, there are no reports of ringed *Myotis* hibernating at underground sites other than where they were ringed.

Figure 4.2. Number of ringed bats of each species recaptured within the same swarming season, and between swarming seasons at Box stone-mine (males and females combined).



4.3.3. Population estimates

The closed capture model (incorporating variability in capture probability over time and individual heterogeneity $\{M_{th}\}$) gave annual estimates of male swarming populations for *M. daubentonii* and *M. nattereri* (Table 4.4). Confidence limits are very large, most probably because the number of recapture events in the encounter history matrices was very small.

Table 4.4. Number of male bats of each species (bold) at the swarming site for each year of study estimated by the closed capture model (M_{th}), with 95% confidence limits (normal type). Where no estimate is given the sample size was too small.

Year	*M. daubentonii* ♂	*M. nattereri* ♂
1999		297 < **1000** < 3765
2000	352 < **713** < 1576	
2001		1109 < **2705** < 6975
2002	293 < **629** < 1501	1482 < **3701** < 9669

Population sizes for male *M. bechsteinii, M. daubentonii* and *M. nattereri* were also estimated for each year from recapture data using the Lincoln-Petersen estimator (Table 4.5). The values calculated by each method of population estimation are similar for *M. daubentonii* and *M. nattereri.*

Table 4.5. Number of male bats of each species (bold) at the swarming site for each year of study estimated by the Petersen-Lincoln method, with 95% confidence limits (normal type).

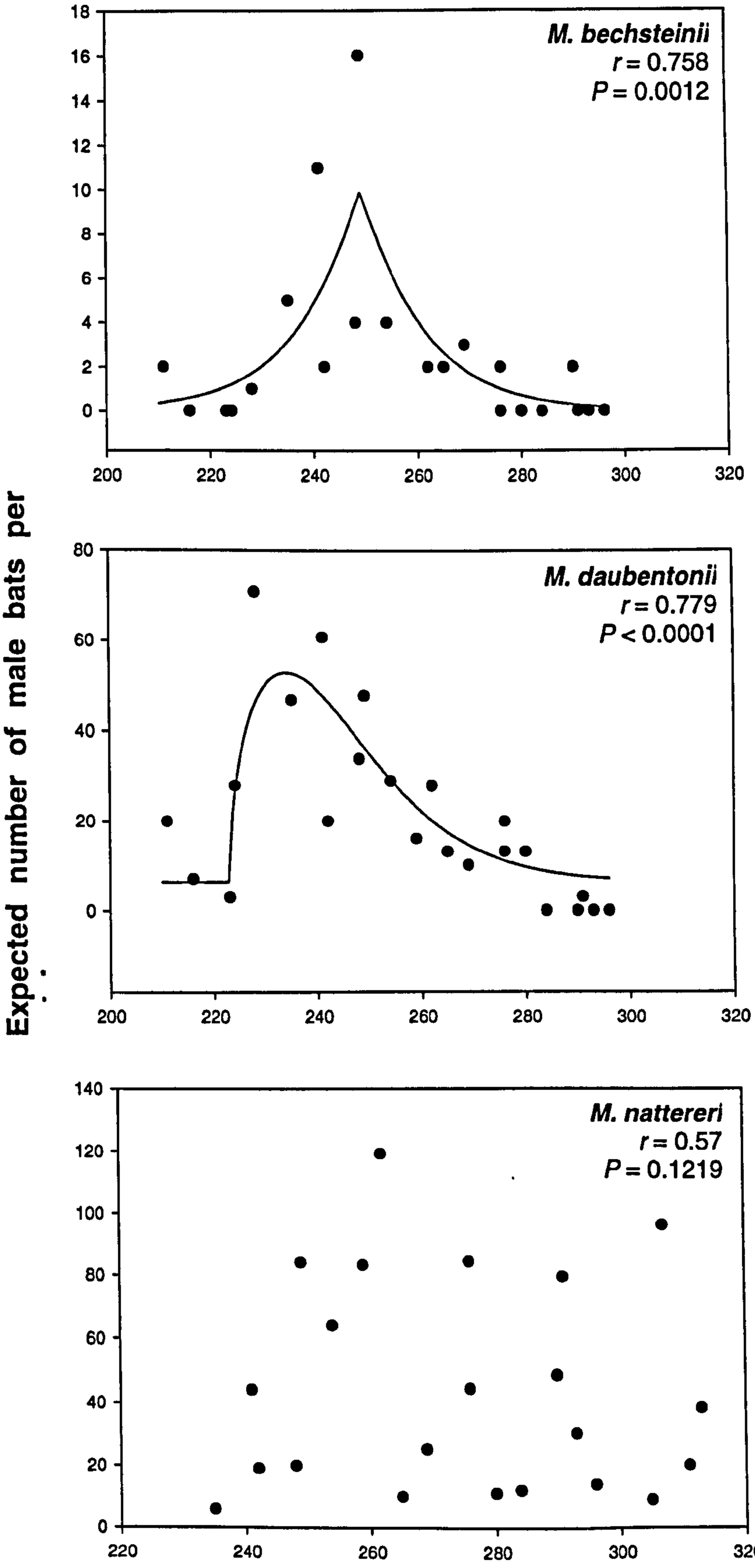
Year	*M. bechsteinii* ♂	*M. daubentonii* ♂	*M. nattereri* ♂
2000	23 < **76** < 146	289 < **741** < 1643	1431 < **4525** < 8612
2001	46 < **154** < 1050	385 < **607** < 1032	1403 < **2316** < 3496
2002	57 < **149** < 674	595 < **837** < 1235	1841 < **2551** < 4225

The sizes of swarming populations were also estimated by extrapolating the nightly male catch for each species (Fig. 4.3) to obtain an expected total number of male bats caught per swarming season. Data for *M. bechsteinii* and *M. daubentonii* are from 29 July to 24 October inclusive, and for *M. nattereri* are from 18 August to 15 November inclusive. The best curve fitted for *M. nattereri* was not an adequate fit ($P > 0.05$). Therefore instead of using the expected total number estimated from the curve, an average expected number of bats caught per night was calculated and used to give a total expected number of males caught per season. Estimates were adjusted down to accommodate bats that were caught but were not novel and are given for three different levels of capture efficiency (Table 4.6). For each method a total estimate of males and females of each species was calculated (Table 4.6).

Table 4.6. Estimated number of males and females of each species (mean (bold) with 95% confidence intervals) visiting Box during the swarming season based on estimates from the closed capture model (Table 4.4) and the Lincoln-Peterson estimator (Table 4.5), and extrapolation from numbers caught at different capture efficiencies.
(Number of females was calculated based on sex ratios given in Table 2.2.)

Species	Closed capture model Mean ± 95% CI	Lincoln-Petersen Mean of 3 years ± 95% CI	Extrapolation at capture efficiency of 30%	75%	100%
M. bechsteinii	N/A	21 < **145** < 269	857	343	257
M. daubentonii	375 < **860** < 1345	565 < **933** < 1301	8276	3310	2483
M. nattereri	431 < **3471** < 7373	167 < **4410** < 8635	18108	7243	5432

Figure 4.3. Curves fitted to expected number of males caught of each species to obtain predicted catch values for each day during the swarming season (29 July to 24 October inclusive for *M. bechsteinii* and *M. daubentonii*, 18 August to 15 November inclusive for *M. nattereri.*) A correlation coefficient (*r*) and *P* value (ANOVA) are given for each graph.



4.3.4. Rate of injury

91.7% of recaptured bats had no evidence of injury caused by the ring. 6.0% (12 individuals) had mild injuries (slight scarring or discolouration of the wing membrane, smoothing of the membrane near the wrist and tiny holes in the membrane near the tips of the ring flanges). No infection was obvious and the rings could always move freely along the forearm, hence it is assumed that these injuries caused little problem to the bat.

2.3% of recaptured bats (4 individuals) had injuries classed as severe. In two bats the ring had punctured the wing membrane and one flange had passed through the membrane to the other side. It was difficult to imagine how this injury had arisen. Some swelling and callused skin was observed at the point of contact with the ring but no infection was obvious. In both cases the ring was removed and replaced on the other arm. In another individual the ring had fused to the membrane and created an open sore on the forearm underneath the main part of the ring. The ring was removed. In the fourth individual, the tip of one flange was fused to the wing membrane but broke free with manipulation and left no hole. In 3.7% of recaptures there was damage or change to the ring. Teeth marks indicated that the bats were perhaps irritated by the presence of the ring. One ring appeared misshapen and another had become orange in colour.

81% of bats recaptured more than once had no injury on both occasions of recapture. Only four bats changed injury status between recaptures; two from mild injury to no injury, one from no injury to mild injury, and one from no injury to severe injury.

To date I know of four ringed *M. nattereri* that were caught by cats. Three were killed but one was released. With this small sample size it is impossible to tell whether presence of a ring has an effect on the ability of a bat to evade capture by a predator, but it is unlikely. One cat also caught another non-ringed *M. nattereri* and where one of the ringed remains was found, remains of numerous other non-ringed animals were also found. One recaptured male *M. nattereri* unfortunately died on the second occasion of being caught. There were no obvious signs of damage, or injury to the bat caused by the ring or otherwise.

4.4. DISCUSSION

4.4.1. Rate of recapture

Between 91 and 99% of recaptured bats were recaptured at the site at which they were initially ringed. Most recoveries or captures of ringed bats away from the point of initial ringing were incidental. Return rate at the swarming sites was overall fairly low (between 6 and 11%) indicating a rapid turnover of animals and a very large swarming population. The recapture rates found for *M. daubentonii* (10.7%) and *M. nattereri* (7.7%) were the reverse of those found by Gaisler and Chytil (2002) for the same species at a swarming site in the Czech Republic (5.1% and 15.5% respectively), although this may be because the distribution of catches during the season was different (Chapter 2).

Return to the swarming site was greater for males than for females for all three species, as found by Navo *et al.* (2002) for *M. lucifugus, M. volans* and *M. evotis* and Fenton (1969) for *M. lucifugus*. From radio-tracking (see Chapter 5) I found variation in the rate of return to the study site between individuals. For example, one male *M. daubentonii* visited every night for three nights, whereas two males and two females returned on only one night. This might suggest that on average male *M. daubentonii* do visit more than females. For *M. nattereri* the only observed return during the night was of a male found active near the swarming site on one occasion. Conversely two males that roosted within 1 km of the site never returned during more than eight days of study. Two female *M. nattereri* used the site as a day roost late in the season. The reasons for visiting may be different for males and females.

Bats of all three species returned more often between successive swarming seasons than within the same season, indicating that few may visit more than once during a swarming season but most return to the same place for swarming in successive years. The proportions of each species caught during swarming in each year that visited more than once (from 0-4.1%) were similar to those found by Fenton (1969) for *M. lucifugus* (from 1.5-4.3%).

Whether capture and attachment of rings or radio-transmitters affects a bats probability of being re-caught is difficult to assess. However activity data (Chapter 3) suggests that activity is not adversely affected by capture and the one male *M. daubentonii* that was radio-tagged and returned to the study site repeatedly, appeared not to have been deterred and has been caught again in the most recent swarming season.

Relatively few ringed bats were seen hibernating at Box and none were found hibernating at the other mine sites. Contrastingly, more ringed bats were seen in hibernation at Savernake Tunnel, possibly because the tunnel has fewer places in which the bats can hide and is less

extensive than the stone mines. Bats have also been actively encouraged to hibernate underneath pieces of wood and Perspex on the tunnel walls where they are easily observed during counts. The finding that bats that swarm at Savernake also hibernate there seems to support the hibernation prospecting theory for swarming, although one ringed bat was found at Savernake that had probably been ringed at Box. Many more years of hibernation survey are needed before the true nature of movement between the different hibernacula is known, but even with many years of study the chances of encountering ringed bats at a site such as Box will remain very small.

4.4.2. Population estimates

The most conservative estimates of population sizes for the three swarming species were from the closed capture model and the Lincoln-Petersen estimator, which gave very similar estimates of population size for each species (around 150 for *M. bechsteinii,* around 900 for *M. daubentonii* and around 4000 for *M. nattereri*). I consider the estimates from the model and Lincoln-Petersen estimator to be more reflective of the actual number of bats present of each species than the extrapolation method. However it should be remembered that the confidence limits for both were large, and so these estimates should also be regarded with caution. The main problem was that a very small proportion of the total population of each species was caught and recapture probabilities were low, which therefore reduces the confidence had in statistical estimators.

The largest estimates were calculated by extrapolation from the number of bats caught during swarming. Data, particularly for *M. nattereri,* may have been skewed by two particularly large catches during fair weather causing the average expected catch to have been over-inflated. It was also difficult to judge what capture efficiency should be applied to the harp traps because it is likely to vary depending on a number of factors. The number of bats present may influence the capture efficiency, for example if many bats detect the trap and circle in front of it other bats may be deterred from approaching. The frequency with which researchers approach the trap to remove captured bats might also affect capture efficiency. On some nights bats may be more frenzied in their flight activity and therefore more likely to be captured than on others, and captured bats may influence the efficiency of the trap by emitting calls that attract conspecifics (Avery *et al.,* 1984; Russ *et al.,* 1998; Thomas *et al,* 1979) which may subsequently also get caught (Greenaway & Hill, 2002).

Bauerová & Zima (1988) stated that their estimate of the size of the swarming community corresponded well with the number of bats hibernating in the region. I am unable to make a similar comparison because counts of hibernating *Myotis* at Box are so low and there are

many other potential hibernation sites within the catchment area. It is impossible to estimate the true number of *Myotis* hibernating in the vicinity. Similarly, so few colonies are known that estimates of swarming populations cannot be compared with the number known from summer roosts. Instead I am able to use published density estimates and knowledge of the extent of the catchment area to estimate how many bats should live within that catchment.

From radio-tracking the catchment area of the study site at Box *M. nattereri* was found to be at minimum 497 km^2 and at maximum 4288 km^2 (see Chapter 5 for further details). Based on an average density of 2.88 bats/km^2 and an average colony size of 35 (Smith & Racey, 2002) and assuming that the entire habitat contained within the areas is suitable for *M. nattereri* these areas could potentially support between 1435 and 12,000 adult females. Assuming an equal sex ratio in the population as a whole, the total number of adults that might inhabit the catchment area of Box would range from 2870 to 24,000 bats. It is difficult to compare this to the national population estimate for *M. nattereri* (Table 1.2) because it is not known how much of the land area of Britain is suitable for and inhabited by the species. It is not unreasonable though to assume that 17,200 km^2 (14%) of Britain's 250,000 km^2 land area is suitable and inhabited, an area that by my estimate would support nearly 100,000 bats.

Assuming a lower population density for *M. daubentonii* of 1 bat/km^2 (Pelikan *et al.*, 1979 cited in Bogdanowicz, 1994; Jones & Altringham, 1996) the catchment area for *M. daubentonii* (254 to 2240 km^2) could potentially support between 254 and 2240 bats. These predicted numbers of bats within the catchment areas are of the same orders of magnitude found by the different methods of population estimates, however must be treated with some caution because it is not known how accurate population density estimates are (particularly for *M. daubentonii* where density is likely to depend greatly on the availability of riparian habitats). Similarly, it is difficult to estimate how much land, nationally, is suitable for this species.

4.4.3. Ring injury and alternatives to ringing

I consider the level of ring injury to be low, particularly when compared to early studies using different types of rings (e.g. Stebbings, 1965 and those cited in Baker *et al.*, 2001), in particular bird-rings and old-type bat-rings. The model of ring used in this study was very different to those described as giving high rates of injury by Baker *et al.* (2001). However, given the total number fitted with rings, 54 bats (2.3% of all those ringed) could have developed a major injury from their rings. It is almost impossible to know whether this injury would confer a greater mortality upon ringed individuals than on those with no ring, partly because of the low rate of recapture and because a different method of marking would have to

be used for those animals that were not ringed so that they could still be recognizable for survival to be assessed. Development of a less harmful alternative to ringing would be welcomed.

PIT-tags (passive integrated transponders) have potential to be of great value in this sort of study, although at present they are expensive and their reliability and injury risk has not yet been fully explored (Baker *et al.*, 2001). PIT-tags have been used in juvenile *M. lucifugus* (T. Kunz, pers. comm.) and in adult *M. bechsteinii* (Kerth & König, 1996) so they should be of appropriate size for use in *M. daubentonii* and *M. nattereri* also. Transponder readers incorporated within the structure of a grill across the entrance to a swarming site, could identify individuals as they enter and leave so a truer representation of the degree of return could be gained than through either infrequent catching or radio-tracking. Similarly by placing transponder readers at roosts used by swarming bats (as found through radio-tracking – see Chapter 5) it could be investigated whether bats were present in the area both before and after swarming. PIT-tagging is not without its risks however. Some catching is still necessary to fit the tags and assessment to ensure no ill effect to the bats is needed. Higher loss or failure rates have been cited for PIT-tags (Baker *et* al, 2001) than for rings therefore the assumption in mark-recapture models that marking is permanent may be violated.

Freeze-branding of bats has recently been suggested as an alternative to ringing (Sherwin *et al.*, cited in Jones, 2002b), however white hair appeared between 22 and 60 days after the freeze brands were applied therefore would be of limited use where bats are re-caught at intervals less than 60 days.

In conclusion, the ringing programme at swarming sites has shown that bats do occasionally return during one swarming season, but they are more likely to be recaptured in a subsequent year or years. Males visit swarming sites more often than females. There is apparently high fidelity to swarming sites during the swarming season and during hibernation. Swarming populations of *M. bechsteinii, M. daubentonii* and *M. nattereri* are estimated as approximately 150, 900 and 4000 respectively, although the reliability of these estimates may be poor due to low recapture rates, variation in activity from night to night and individual heterogeneity in capture probability.

CHAPTER FIVE

DISPERSION AND HABITAT USE BY *M. DAUBENTONII* AND *M. NATTERERI* DURING THE SWARMING SEASON

5. DISPERSION AND HABITAT USE BY *M. DAUBENTONII* AND *M. NATTERERI* DURING THE SWARMING SEASON [1]

SUMMARY

24 *M. daubentonii* and 35 *M. nattereri* were fitted with radio-transmitters during the swarming season to determine the catchment area of the swarming site and to study habitat use and nightly activity of individuals during the swarming season. Overall 61% were located after release and 39 day roosts were found for 31 individuals.

Maximum range from the swarming site was 26.7 km for *M. daubentonii* (mean (± SD) 18.7 ± 9.4 km) and 24.8 km for *M. nattereri* (mean 12.0 ± 8.0 km). Minimum convex polygons depicting the minimum catchment area of the swarming site measured 254 km^2 for *M. daubentonii* and 497 km^2 for *M. nattereri*. Including recoveries of ringed bats increases the area enclosed by the maximum range to at least 4118 km^2.

Distribution of day roosts was skewed towards the south and east in a non-random manner for *M. nattereri* and to the south for *M. daubentonii*. Compositional analysis of habitat used in relation to that available suggested selection of mixed agricultural areas in which to roost by *M. nattereri*. Broad-leaved woodland was preferred for foraging by this species. Parkland, woodland and open water habitats were common around roosts of *M. daubentonii*. Such habitats should be conserved and enhanced to protect these species.

Bats of both species were faithful to small home ranges (<3.4 km^2) and rarely travelled further than 3 km from their day roosts during the night.

Some individuals of both sexes returned to the site of capture, but none visited another swarming site. Thousands of *M. daubentonii* and *M. nattereri* gather at swarming sites from many colonies distributed over a large area. Such sites are probably important centres of outbreeding, which maintains genetic diversity in populations. Bats may be faithful to only one site and may be inflexible should that site be destroyed. Protection of these mating sites should be a priority.

[1] A paper based on some of the data in this chapter has been accepted for publication in Animal Conservation with the title 'Dispersion and habitat use by *Myotis daubentonii* and *Myotis nattereri* during the swarming season: implications for conservation'. G Jones is co-author.
See Appendix 3 for copy of proof.

5.1. INTRODUCTION

To date primary investment in UK bat research has investigated summer roost requirements and reliance on underground sites for hibernation (Hutson, 1993). Little is known about the whereabouts and activity of males and females of most bat species between weaning of the young and the onset of hibernation. It has been presumed that bats disperse in the environment and for the most part live solitarily or in small groups at this time. Most colonies break up several months before hibernation is considered to begin. Davis and Hitchcock (1965) concluded that *M. lucifugus* "wander considerably" after the break up of the maternity colonies and that some movements suggested "random wandering". We now know that some bats visit swarming sites during this time but we do not know from how far they travel, whether they return to familiar roosts and feeding areas after swarming and how often they visit swarming sites. These questions can be tackled with radio-telemetry.

5.1.1. Delineating the catchment area of a swarming site

In Chapter 4, I hypothesized that the catchment area of a swarming site is likely to be very large, by virtue of the number of bats visiting the site. Few bats reside at swarming sites during the day (Davis & Hitchcock, 1965; Fenton, 1969; Hall & Brenner, 1968; Harrje, 1994; Humphrey & Cope, 1976; Whitaker & Rissler, 1992), so I aimed to discover where they roost and consequently estimate the size of the catchment of the swarming site. Knowledge of the locations of roosts and colonies is of vital importance for their protection.

Marking animals by ringing can contribute information about their distribution away from the point of ringing (Davis, 1964; Davis & Hitchcock, 1965; Bauerová & Zima, 1988). In North America, bats ringed at swarming sites have been recorded up to 483 km away on their summer ranges later in the same season (Davis, 1964). However, unless many thousands of bats are ringed this technique generally results in few recaptures over time, especially where knowledge of extant summer roosts is scarce.

The most appropriate method for delineating the area from which bats are drawn to a specific swarming site is radio-telemetry. Study animals are fitted with a transmitter emitting a pulse of known radio-frequency, which can be followed using radio-receiving equipment. Day roosts can be pinpointed by homing-in on the signal. This is the first study to radio-track bats from a swarming site and the first to track bats intensively during the gap between summer and winter roosts. It is also one of the few studies to radio-track a large number of male bats. The majority of prior studies have tracked females from nursery colonies (e.g. Catto *et al.*, 1996; Clark *et al.*, 1993; Henry *et al.*, 2002; Kerth & König, 1999; Seimers *et al.*, 1999).

Inevitably the catchment area has to be estimated by the movement of bats away from the site, rather than coming to the site. It may appear problematic to define a catchment area by where bats go to having visited a swarming site, but it is viable if bats are found on the same ranges before swarming, as well as afterwards (suggested by Clark *et al.*, 1993; Davis, 1964; Furmankiewicz, 2002 and author pers. obs.). Female ranges (aka 'familiar areas') may be bigger than those of males because males do not have to travel so far from winter hibernacula to find summer roosts, whereas females may require specific roosts for parturition and lactation (Humphrey & Cope, 1976). Conversely, it has also been suggested that males travel further than females because of natal dispersal and they may visit many different swarming sites in the search for mates.

5.1.2. Roost distribution and habitat choice

When roosts were distributed in a particular area I asked what features of the habitat might be suitable for that species. Habitat choices can be investigated on several scales. At a large-scale, I asked why bats live in a particular region and not in another by comparing habitat around roosts with that around random points (Oakeley & Jones, 1998). At a smaller scale, I investigated use of habitat on a nightly basis for foraging by comparing habitats used within the home range with those habitats available around the roost. The home range is defined as the area normally used by the animal on a daily basis. From previous studies (Glendell & Vaughan, 2002; Rydell *et al.*, 1994; Seimers *et al.*, 1999; Smith & Racey, 2002; Swift & Racey, 1983; Vaughan *et al.*, 1997), I predicted that *M. daubentonii* would forage predominantly in riparian habitats and *M. nattereri* in broad-leaved woodlands, along tree-lined corridors and over grassland. Knowledge of habitats used on the large and small scales by the bats will be of benefit to the statutory nature conservation organizations in designating areas of conservation priority and in planning future landscape enhancements for bats.

5.1.3. Nightly activity budgets

Nightly activity budgets of bats during the swarming season might include foraging, commuting, swarming and night roosting. Time of emergence in bats is related to the time of sunset. Previously published average emergence times for the two species are 56 minutes after sunset for *M. nattereri* (Swift, 1997) and between 45 and 85 minutes after sunset for *M. daubentonii* (Jones & Rydell, 1994; Richardson, 1985). Time of return to the roost may be connected to time of sunrise, although the time spent roosting during the night might increase later in the season when fewer insects are available and temperatures are colder.

5.1.4. Return to swarming sites

Ringing can provide only limited data on return to a swarming site, unless captures are made every night, as by Davis and Hitchcock (1965). However, this constitutes great disturbance and bats may become trap-shy if traps are used on consecutive nights (Duffy et al., 2000; Kunz & Anthony, 1977) and hence become even less likely to be re-caught even if present in the area. Low visitation of the swarming site is predicted from mark-recapture studies (Hall & Brenner, 1968; Harrje, 1994; Whitaker & Rissler, 1992). However if mating and mixing of genes are important functions of swarming we might expect bats to return to find new mates or to visit other swarming sites to promote genetic mixing (Davis & Hitchcock, 1965). Return rate was given for ringed bats in Chapter 4 and additional data from radio-tracked bats are added here.

Movements to other swarming sites can be monitored by ringing and radio-tracking. There are at least ten other disused underground sites within 10 km of the study site, two of which are monitored by catching surveys during swarming. Longer distance movements might be detected by catching at other sites, by bat workers monitoring bat boxes and by finds of marked bats by members of the public.

5.1.5. Study species

M. daubentonii and *M. nattereri* were chosen for radio-tracking because they are most common at the site (Chapter 2) and they are the subject of population estimation work through mark-recapture (Chapter 4). A study of two species should provide a broader view of the system than the use of only one species by permitting comparisons to be made. Historically both species have had a scattered distribution in Wiltshire, although the level of recording has not been consistent throughout the whole county (Dillon, 1997). Most records are from underground sites during hibernation, but both species use trees as day roosts during the summer, which are difficult to locate. *M. nattereri* also use buildings, particularly as parturition and nursery roosts. However prior to this study only one colony was known in the study area.

I predict that *M. daubentonii* might travel further and consequently have a greater catchment area, because it has flight morphology (higher wing loading hence greater flight speed and less manoeuverability) that is more suited to longer distance movements (Norberg & Rayner, 1987), and secondly, because it is connected with riparian habitats (Swift & Racey, 1983) and hence may require a greater range to reach suitable habitat.

The specific aims of this chapter are:

1. To delineate the minimum catchment area of a swarming site by locating day roosts of *M. daubentonii* and *M. nattereri.*
2. To describe and explain observed distributions of both species.
3. To examine habitat within home ranges and within foraging areas for each species.
4. To record nightly activity budgets, including time of emergence from and return to the roost, time spent foraging and time spent swarming.
5. To quantify rate of return to swarming sites and movement to other swarming sites.

5.2. METHODS

5.2.1. Capture of bats and attachment of radio-transmitters

The study site was Jack's entrance to Box limestone mine as detailed in Chapter 2. Between four and eight bats were fitted with radio-transmitters (Model LB-2, Holohil Systems Ltd., Ontario, Canada) on each of nine catching events between August and October in 2000 and 2001 (Appendix 5). In total 59 bats (24 *M. daubentonii* and 35 *M. nattereri*) were radio-tagged. Bats were selected either at random or in sixteen cases because they had previously been caught at the site. In one case a bat was radio-tagged in both years. More males (36) were tagged than females (23) to partly reflect the highly male-biased sex ratio during swarming. *M. daubentonii* were tagged earlier in the season (August and September) when they were present in greater numbers, and *M. nattereri* later in the season (September and October).

Transmitters were attached dorsally between the shoulder blades (Plate 5.1). A small patch of fur was clipped using scissors and the transmitter attached using SkinBond surgical adhesive (Smith & Nephew, supplied by Canada Care Medical, Ottowa, Canada) which remains flexible when dry (Swift, 1998) and has been found to be harmless unlike epoxy glues (Duvergé, 1996). The transmitters weighed on average 5.90 ± 0.57% (mean ± SD) of total body weight on *M. daubentonii* and 6.45 ± 0.63% on *M. nattereri*. In 2000, lighter weight (0.48g) shorter duration (10-day) transmitters were used for *M. daubentonii*, and heavier (0.52g) longer duration (21-day) transmitters were used for *M. nattereri*. In 2001 the transmitters used on *M. daubentonii* weighed 0.52g, but were of reduced battery duration (14-days) and increased range in an attempt to increase success in locating tagged bats. The remainder of transmitters used in 2001 weighed 0.52g and had battery duration of 21-days. (See Appendix 5 for more information on transmitter masses and bat masses).

5.2.2. Radio-tracking equipment

Bats were located after release by using Lotek radio-receivers (Models SRX_400 and STR_1000, Lotek Engineering, Ontario, Canada) and a combination of aerials (Biotrack, Hants, UK) including, magnetic whip aerials for attachment to the car roof, three-element flexible and collapsible Yagi aerials and a rigid six-element Yagi aerial for use mounted on a pole or through the sun-roof of the car (Plate 5.2). This aerial could be rotated through 360 degrees and was mounted up to a maximum of five metres above the ground on interlocking one-metre lengths of aluminium tubing. Increasing the height of the aerial above the ground greatly increased the range over which signals could be detected (maximum achieved was 12.5 km from one high point to another).

Due to poor success in finding tagged bats in 2000 by ground searching, I decided to employ surveys from the air to enable coverage of a greater area more quickly and more thoroughly. This is particularly advantageous when simultaneously searching for multiple animals (Hicks *et al.*, 2001), which may have dispersed in any direction from a central release point. Adrian Warren (Last Refuge Aviation Ltd, Somerset, UK), a Cessna pilot with prior experience of radio-tracking was engaged for an initial flight of one hour 55 minutes in October 2000 to assess the method. Cessna are considered ideal for radio-tracking (Kenward, 2001) because the high wings supported by struts provide an excellent position for the mounting of receiving aerials. For this flight one half of the six-element rigid Yagi aerial was attached to a clamp usually used for mounting a camera to the wing strut of the plane (Plate 5.3a). This attachment method was not ideal, although four out of nine bats with transmitters were located (one bat was known to be roosting underground at the time hence I consider the success of this trial to be 50%).

For reasons of safety and survey effectiveness it was necessary to develop equipment specifically for the plane. Custom-built clamps were made for the attachment of a radio-aerial to each wing strut of the Cessna (Opt Out Engineering Ltd., Bristol, UK). The initial design was suggested by Kenward (2001), but developed according to that used for a camera mount, previously manufactured for the same plane by the same engineers. A rigid three-element Yagi aerial was secured in each clamp angled toward the ground at approximately 30 degrees from the horizontal (Plate 5.3b). Greater range may have been achieved by mounting the aerials in a vertically polarized manner rather than horizontally (Kenward, 2001), however in the interests of aero-dynamics, and consequently safety, the pilot preferred horizontal polarization.

When surveying in the plane each aerial was attached to a Lotek radio-receiver and the output from each was fed into each side of a pair of closed audio headphones via a connecting box (Kenward, 2001). The Loteks were set to scan the radio frequencies of the tagged bats with a scanning interval of 4 seconds.

5.2.3 Air search methodology

When animals can have dispersed in any direction from a central point it is best to search outwards from the release site (Kenward, 2001). Therefore during the search phase of flights we flew an outward anti-clockwise spiral from the point of release. A test flight on 29 July 2001 demonstrated that a transmitter could be received over a distance of approximately 7 km at a height of 1000 m. The material of the roost (stone building, tree, wooden barn etc) may

affect signal transmission, so we decided that each spiral should be approximately 5 km distant from the previous one to minimize the chance of missing a signal.

When a radio-signal was received the plane was turned in circles or figures of eights over the area until the strongest signal revealed an area on the ground where the transmitter was. This method of homing in on the signal was deemed more appropriate for this study than either the recording of signal strength on successive flight paths or triangulation by taking bearings. On occasion a signal was first received from over 10 km away indicating the great advantage of aerial searching for boosting the range of signal reception.

In total, sixteen and a half hours of searching were conducted from the air and each flight covered an area of approximately 3000 km^2. During the first search flight one aerial malfunctioned (cause unknown) and so search efficiency was reduced (the fewest number of bats was found on this flight). On another flight one receiver battery ran down, but with only half an hour remaining of the three-hour flight.

Ideally ground searches of all areas identified during flights would have been made on the same day as the flight. This 'ground-truthing' was unfortunately not possible because no other tracking teams were available. Except on one occasion, searching on the ground in the areas identified during the flight was done on the following day. One disadvantage of air searching is that animals roosting underground, for example in the mine, are not detected (Stebbings, 1982). The study site should have been searched prior to the flight to eliminate any bats found roosting there from the air search. Again, with the exception of one occasion this was not possible due to constraints of both time and manpower.

5.2.4. Ground search methodology

Before the introduction of air searches ground search protocol was to search the area around the study site as thoroughly as possible using available roads and footpaths. High points were used to give a good view over the surrounding area. Searches began close to the study site and gradually moved away. At all times when driving in the area an aerial was on the roof and the receiver was scanning for the active transmitters. Searches were conducted both during the day for day roosts and during the night for active bats. Day roosts were located by homing in on the signal and their positions were recorded as an eight-figure grid reference. In densely wooded areas it was often difficult to pinpoint an exact tree as the roost, especially if no tree cavities were obvious. When roosts were in buildings I could usually determine the entrance/exit point of the roost by homing on the strongest signal and there was usually a visible hole, sometimes with staining around the entrance.

In total 575.5 hours was spent searching for and listening to radio-tagged bats on the ground. For 66 hours two teams were searching simultaneously. For the remainder one team was out alone. Sometimes the second team was stationed outside the mine entrance to wait for visiting bats. A scanning receiver with logging facility could have been left at the mine system permanently during the project to monitor returning bats, however this was not possible due to the risk of theft of equipment. In total across the two seasons 79 days of tracking were performed giving an average time in the field of 7.28 hours per day.

5.2.5. Data collection

Locations of bats (fixes) were recorded continuously from the time the bat emerged or the time it was found, until the time the bat returned to the roost or when contact was lost. If contact was lost, foraging areas, roost areas and the release site were searched. Usually one bat was followed continuously per evening. Occasionally, where tagged bats were close to one another I could monitor two bats at once by alternating between the frequencies.

Most fixes were obtained by the close approach method of homing in on the signal to locate the bat. However because of the dark and the mobility of the subject the bats were rarely seen during tracking. Where close approach was not possible (for reasons of access and safety), bearings were taken using a compass and distance to the bat was estimated from signal strength readings at different gains, knowledge of the terrain and observer experience (O'Donnell, 2000). Distance estimation was checked by using a transmitter placed in the field at known distances from the observer. When a bat was stationary for a length of time, triangulation was possible by moving between two or three points and taking bearings. This was a necessary method in locating night roosts when access to land had not been arranged and also in locating bat D18, which roosted within the safari park at Longleat and hence could not be approached on foot for fear of attack by lions!

Six-figure grid references were recorded for the locations of the bats (±100 m) and plotted on Ordnance Survey 1:25000 maps. When noting fixes, day roosts were recorded twice (once in the evening and once on return). Although location and activity of the bat were recorded continuously during tracking, 15-minute fixes were used in calculation of home range to avoid the problem of autocorrelation (O'Donnell, 2000). At night a bat was recorded as active if the signal strength was variable. Once the signal went stable the bat was considered stationary and attempts were made to locate (or triangulate) the night roosts. The time of emergence and return to the roost was noted and on some occasions the roost was watched at emergence with or without the aid of an infra-red video camera and/or a bat detector to count the number of emerging bats.

5.2.6. Data analysis

Radio-tracking data were displayed and analysed with the program Animal Movement (Hooge & Eichenlaub, 1997), an extension of ArcView (ESRI GIS and Mapping Software). Movement data from captures and finds of ringed individuals at locations other than the release site are included also (for details of bats ringed see Chapter 4). Base maps (Strategi 1:10,000 and Landline 1:1,2500) were obtained from Digimap (© Crown Copyright Ordnance Survey. EDINA Digimap/JISC) and converted for use in ArcView with Map Manager (ESRI UK). Patterns of distribution of day roosts around the study site were analysed using circular statistics (Batschelet, 1981; Zar, 1974).

The 'study area' is defined as a circle, with the release site at centre, containing all of the fixes of the radio-tagged bats. 'Catchment area' is defined as the area from which bats were drawn to the study site. Minimum catchment areas for each species were represented by connecting the outermost day roosts of each species to form minimum convex polygons (MCPs). Potential catchment areas for each species were delineated by drawing maximum range circles (MRCs) with the study site at centre and radius of the furthest day roost found from the study site for each species.

'Home range' is defined as the area normally used by the bat on a nightly basis, and does not include the study site unless visited after release. Had the study site been included, particularly in construction of 100% MCP home ranges, a large amount of space would have been included that was never used by the bats. I consider it more accurate to describe the 'local' home range of each bat, assuming that they make occasional direct flights to and from the study site for the purpose of swarming. The home ranges are therefore seasonal and cannot be expected to describe the annual range requirements of these animals (Harris *et al.*, 1990). Instead I expect to obtain a reasonable estimate of the home range the animal is using in the short term (Kenward, 2001).

In addition to 100% MCP home ranges, I constructed Utilization Distributions (UDs) following the Kernel method to remove areas little used by the bats and to describe core areas of activity (usually feeding patches). 80% and 50% UDs were constructed in this way, providing contours of 80% and 50% probability of the bat being within those regions. Plots of increase in home range size with increasing sample size were constructed to ensure that asymptotes had been reached (Harris *et al.*, 1990).

A large scale overview of the landscapes available in the study area was supplied by the land class database held by the Centre for Ecology and Hydrology at 1 km resolution (Bunce *et*

al., 1996); however, this distinguished only arable from pastural landscapes. For a more detailed analysis of habitats 'available' and 'used' by the bats, habitat within 2 km of the day roosts of radio-tagged bats and within 2 km of 20 points selected randomly from within the study area was mapped and digitized in ArcView. The mean furthest distance traveled from the roosts in the 80% UD for home ranges that were well revealed for both species was 1.6 km (n=4) for *M. daubentonii* and 1.9 km (n-19) for *M. nattereri.* To maintain statistical independence of data only one roost was mapped per individual. Where an individual had more than one roost, the one that was utilized more was mapped. In all cases the other roosts (n=9) were within the 2 km radius also. All individuals had to be considered independent from one another (i.e. not part of a group) for compositional analysis (Aebischer *et al.*, 1993). This was possible because no two animals shared the same roost, despite the proximity of some, which may perhaps indicate associations during other seasons.

Random grid references were generated using the 'random numbers' function in Excel. Circular statistics confirmed that the random points were randomly and uniformly distributed around the release site ($R = 3.35$, $P > 0.50$; $\chi^2 = 13.59$, d.f. = 19, $P > 0.75$). Therefore 11.22% of the study area was mapped. This method was used because it was not possible to map the habitat within the entire area due to its large size.

A small amount of Phase I habitat survey data was supplied by Bristol Regional Environmental Records Center and the Wiltshire and Swindon Biological Records Center, but for much of the area such data was unavailable. Mapping was therefore carried out by field visits supplemented by information from aerial photographs (www.Multimap.com). Eight habitat categories were considered: Amenity (grass airfields and playing fields), Arable, Parkland (including large gardens), Pasture (meadows and grazed pasture), Open Water, Scrub/Heath, Urban (rural, suburban and urban built-up land) and Woodland (91% deciduous).

Statistical analyses comparing habitat available with the habitat used were performed with Compositional Analysis Excel tool 3.1 written by Peter Smith (University of Aberdeen) according to the methods of Aebischer *et al.* (1993). See Russo *et al.* (2002) for application to bat radio-tracking studies. Default settings were used and 0 values were replaced with 0.0001.

Times of official sunset and sunrise were obtained from Whitaker's Almanac (Hill, 2000; Hill, 2001).

Plate 5.1. (above) *M. nattereri* with radio-transmitter attached

Plate 5.2. (left) Taking a bearing on a radio-signal to home in on the day roost of a tagged bat. The tall mast and six-elements of the aerial served to greatly increase the range of reception.

Plate 5.3. (a) Original aerial position for the trial flight in 2000.

Plate 5.3. (b) One of the aerials in position for flights in 2001. Clamps could be adjusted to alter the angle of the aerials relative to the ground.

5.3. RESULTS

5.3.1. Success rates

36 (61%) radio-tagged bats were relocated again after release (Appendix 3). Data on five individuals were discarded due to transmitter frequency overlap with other radio-tagged animals in the area (water voles (Plate 5.4) and grey partridge). More *M. nattereri* were relocated (68.6%) than *M. daubentonii* (50.0%). Find rate was particularly poor for *M. daubentonii* during 2000 (14.1%). There was no difference in relocation rate between males (61.0%) and females (61.0%) for either species. 39 day roosts were found for 31 individuals and sufficient data (i.e. asymptote had been reached) were collected for 13 individuals of *M. nattereri* and four individuals of *M. daubentonii* to determine home ranges and study their activity. Mean contact time with an individual was 4.2 nights (min < 1 night, max 10 nights).

Signals from 25 (52.1%) of the 48 transmitters that were active during flights were heard from the air. Twenty were subsequently located on the ground, usually within one kilometer of the location that was suspected from the air. On occasion they were found to be in exactly the same location (group of buildings or area of wood) as suspected from the air. Five transmitters heard from the air were never found on the ground (D13, D15, D23, N29 & N30). The most likely reason is that the bats had moved between the flight and the ground search. Alternatively, the transmitter may have failed or the signal might have been masked on the ground. These locations are not included in the detailed analysis. Eight radio-tagged bats that were not heard during plane searches were later found within the study area. Possible explanations include: (i) the bat may have been in the area but was missed for some reason, for example during the plane search another bat in the same area was heard and scanning was stopped to pin-point the first bat and the second bat was missed but later picked up during ground searching (e.g. N18), (ii) the bat may have been underground (e.g. N13, N35), (iii) the bat may have been in a narrow valley and/or in a solid building which reduced the signal range (e.g. N10) or (iv) the bat may have been outside the area searched and later moved inside.

Four tagged bats (D11, D14, D19 & D20) were not heard during the flights but were found near the release site during night-time ground searches. They were not found underground during the day, but they may have roosted deeper in the mine than was searched. Alternatively they may have traveled in from outside the plane search area. It should be noted that for some frequencies (including that of bat N18) the level of interference was especially great which made listening to and detecting the signal very difficult.

The majority of transmitters remained attached to the bats until the batteries failed. Two transmitters were recovered having become detached from the bats 17 and 16 days after attachment. Several others may also have become detached but I was not able to reach them to confirm this. In the final batch of transmitters in 2001, when weather was beginning to get cold, bats may have entered torpor, which might explain the lack of movement of N32 and N35.

To date three *M. nattereri* and five *M. daubentonii* that were radio-tagged have been caught again. These animals had all survived for at least one year after tagging, including the hibernation period. When bat D10 was recaptured in April 2002 it was heavier than in August 2001, although a bare patch was still discernible where the transmitter had been attached. Bats may be unable to replace lost hair during hibernation while their metabolism is reduced. A cat caught bat N12 just over one year after tagging and its hair had grown back fully. Similarly bats D6, D8, D11, D17, N5/N29 and N14 showed no evidence of having been tagged when re-caught at the release site in subsequent swarming seasons.

5.3.2. Distribution of day roosts

Day roosts were found for 23 *M. nattereri* (30 roosts) and eight *M. daubentonii* (nine roosts) (Fig. 5.1). Average distance of day roosts from the release site was 14.36 ± 9.20 km (mean ± SD) for *M. daubentonii* and 12.49 ± 8.49 km for *M. nattereri* but this difference was not statistically significant (t = 0.67, d.f. = 10, P = 0.52). Neither were there significant differences between males and females of the two species in distance of roost from release site (*M. daubentonii:* W = 17.0, N_1 = 5, N_2 = 4, P = 0.37; *M. nattereri*: t = 0.41, d.f. = 13, P = 0.69). The maximum distance traveled from the release site to a roost was 26.7 km, which was recorded for a female *M. daubentonii* (D22).

Nine bats used two roosts during observation. Average distance between different roosts of the same individual was 0.78 ± 0.49 km for *M. daubentonii* (n=2) and 0.66 ± 0.51 km for *M. nattereri* (n=7). The closest were only 0.10 km apart and the furthest 1.53 km. No bat was observed to use more than two roosts although there were occasions when a bat was absent from previous day roosts and therefore must have roosted elsewhere. Difficulty arose when I was unable to determine whether the bat had actually left the area or if the transmitter battery had failed.

MCPs constructed by joining all day roosts of each species measured 253.73 km^2 for *M. daubentonii* and 497.08 km^2 for *M. nattereri* (384.44 km^2 without a roost found only from the plane) (Fig. 5.1). Maximum Range Circles (MRCs) calculated from the radius of the furthest

day roosts from the release site have areas of 2239.61km^2 for *M. daubentonii* and 1932.21km^2 for *M. nattereri*. However it should be noted that these outer day roosts were nearly at the furthest extent of the air searches, and so bats beyond this area would not have been relocated. These figures should therefore be regarded as minima. There were no apparent differences between males and females in distribution. Day roosts for both species were neither randomly nor uniformly distributed around the release site, instead they were skewed to the south for *M. daubentonii* (mean angle from North = 189° ± 018°) and to the south-east for *M. nattereri* (mean angle = 128° ± 005°) (Fig. 5.2).

5.3.3. Movement of ringed bats

Four *M. nattereri* and one *M. daubentonii* ringed at the study site were relocated elsewhere (Fig. 5.3). One *M. nattereri* (assumed though not confirmed to be from Box) was seen during hibernation at Savernake Tunnel (36.1 km from Box mine). Another was caught by a cat in Downend, on the outskirts of Bristol (20.8 km), in November 2001. A third ringed *M. nattereri* was also caught by a cat only 4 km from the study site and a fourth, a female, was caught by me during early summer at a roost used by one of the radio-tagged bats, and later found to house a sizeable maternity colony. One male *M. daubentonii* ringed at the study site was caught during a routine survey by Wiltshire bat group close to Savernake Tunnel during the swarming season in 2002. Including recoveries of ringed bats increases the area enclosed by the maximum range to at least 4118 km^2.

In addition to movements from the swarming site to other places, there are records of bats ringed elsewhere and later caught at the release site during swarming (Fig. 5.3). A female *M. nattereri* ringed at a maternity colony to the west of the mine and a female from the Savernake Forest colony were caught at the mine during swarming in September 2002. A male *M. nattereri* caught in Savernake Forest in August 2002 was caught at the mine just over one month later. Distances traveled by ringed bats from other swarming sites also fit within these ranges (e.g. from Chilmark to Salisbury = 16.2 km and from Fonthill to Ringwood = 28.1 km).

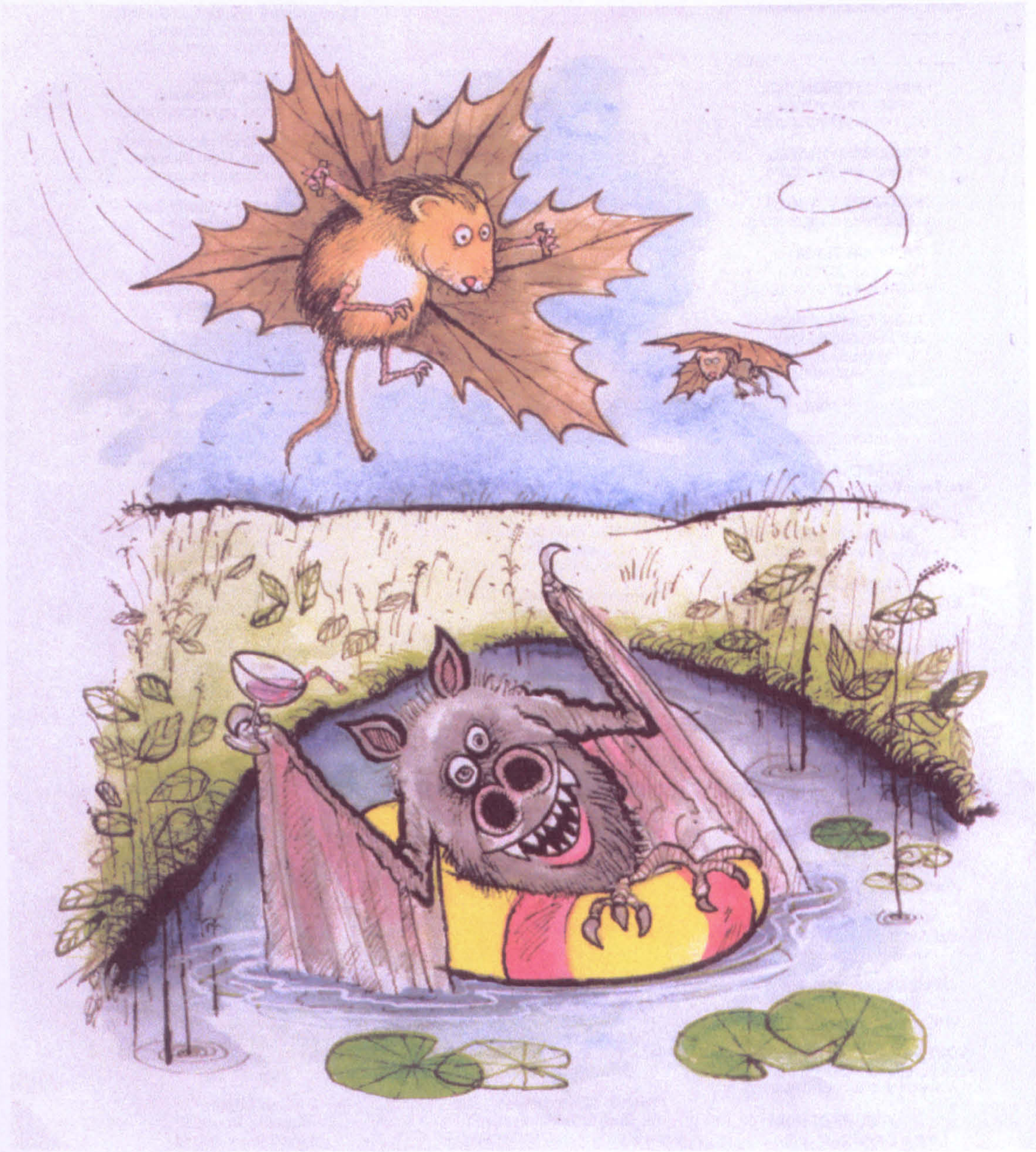


Plate 5.4. Cartoon depicting 'flying voles' and 'swimming bats'.
By Mark Brewer for Wildlife Conservation magazine, October 2001.

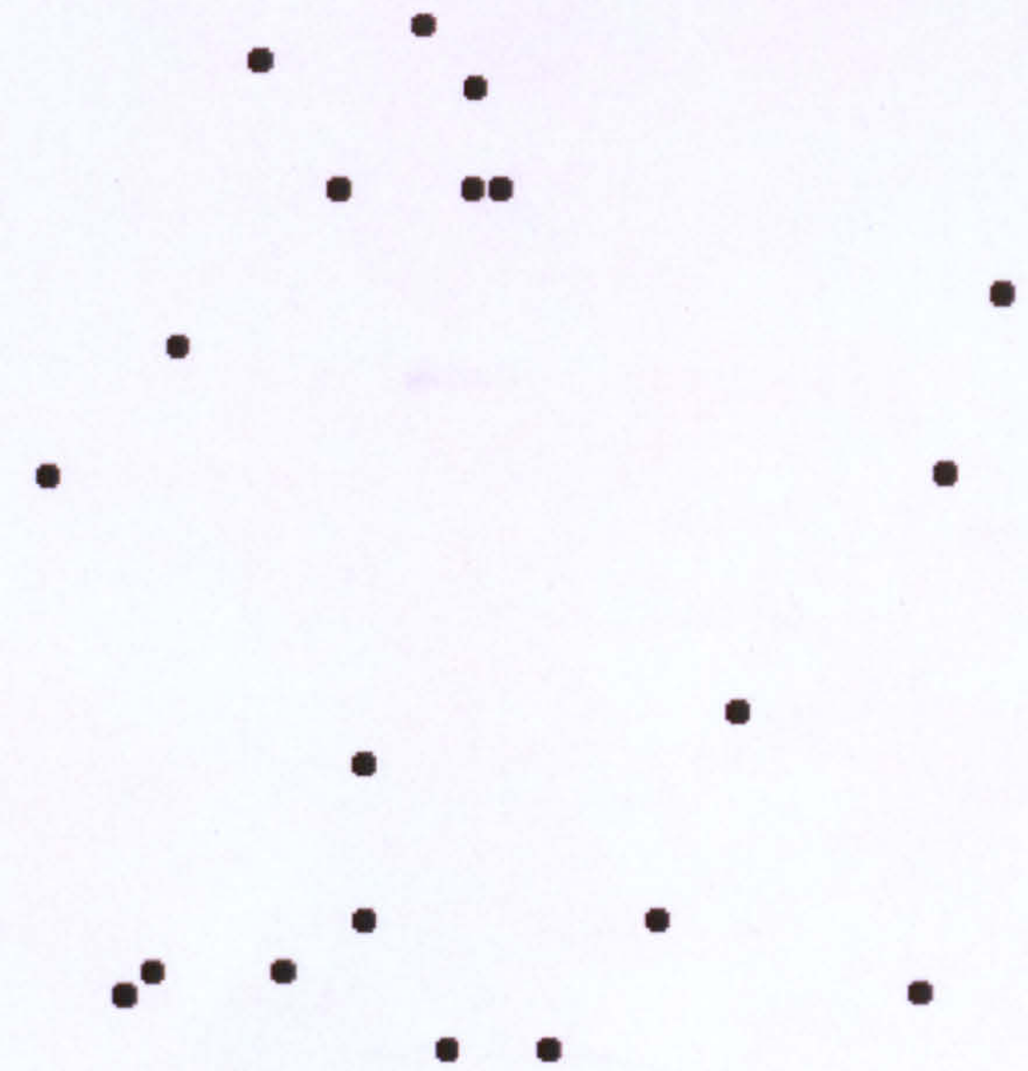


Fig. 5.1 overlay.
Random points used in comparison of habitats around roosts with a random sample.

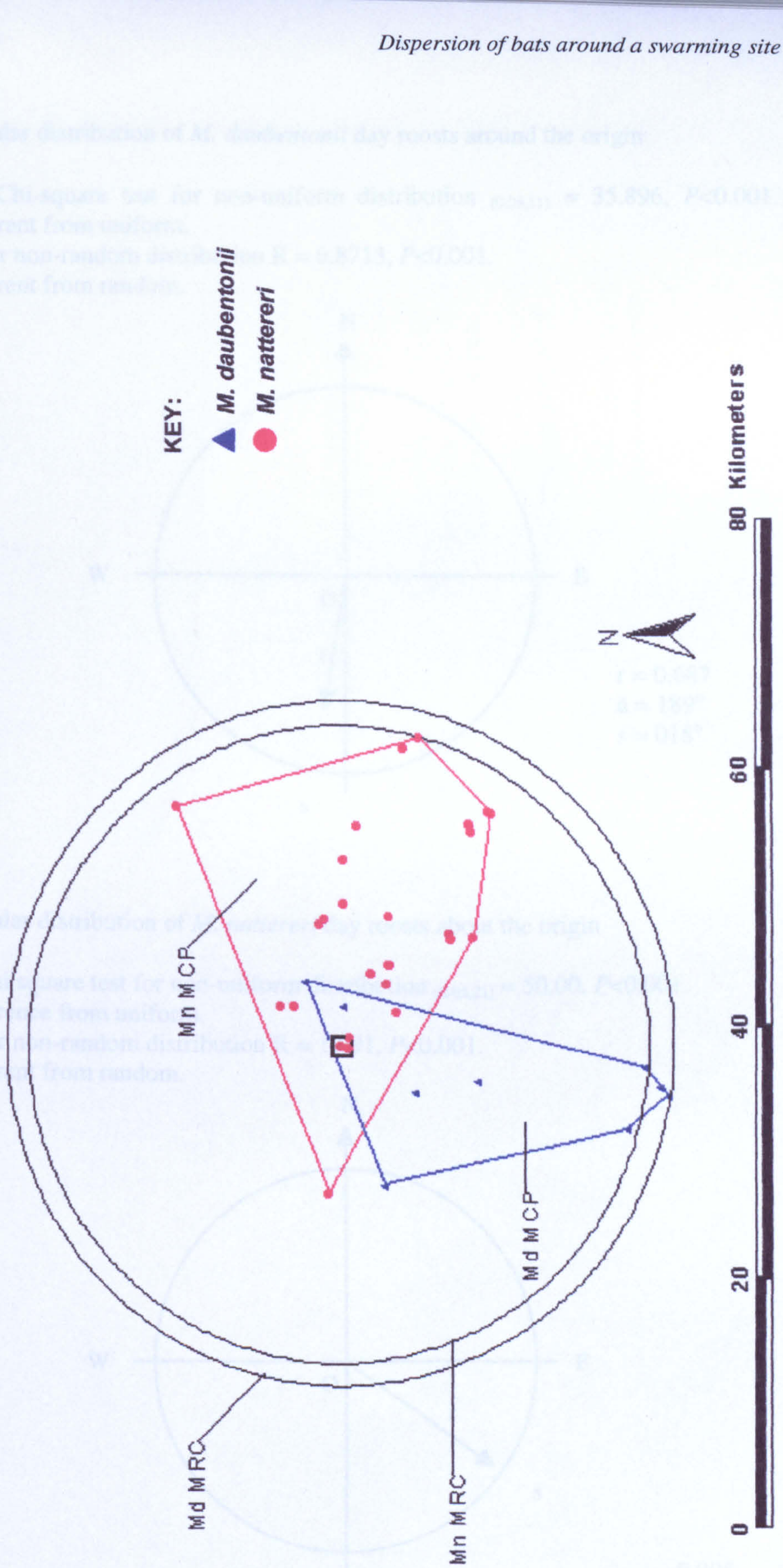


Figure 5.1. Locations of day roosts of eight *M. daubentonii* and 23 *M. nattereri* relocated by radio-tracking between August and November, 2000 and 2001. Outermost day roosts of both species have been joined to give Minimum Convex Polygons (MCPs) and the furthest radius of each species has been used to construct Maximum Range Circles (MRCs). The release site is at center. Md = *M. daubentonii*, Mn = *M. nattereri*.

Figure 5.1. Locations of day roosts of eight *M. daubentonii* and 23 *M. nattereri* relocated by radio-tracking between August and November, 2000 and 2001. Outermost day roosts of both species have been joined to give Minimum Convex Polygons (MCPs) and the furthest radius of each species has been used to construct Maximum Range Circles (MRCs). The release site is at center. Md = *M. daubentonii*, Mn = *M. nattereri*.

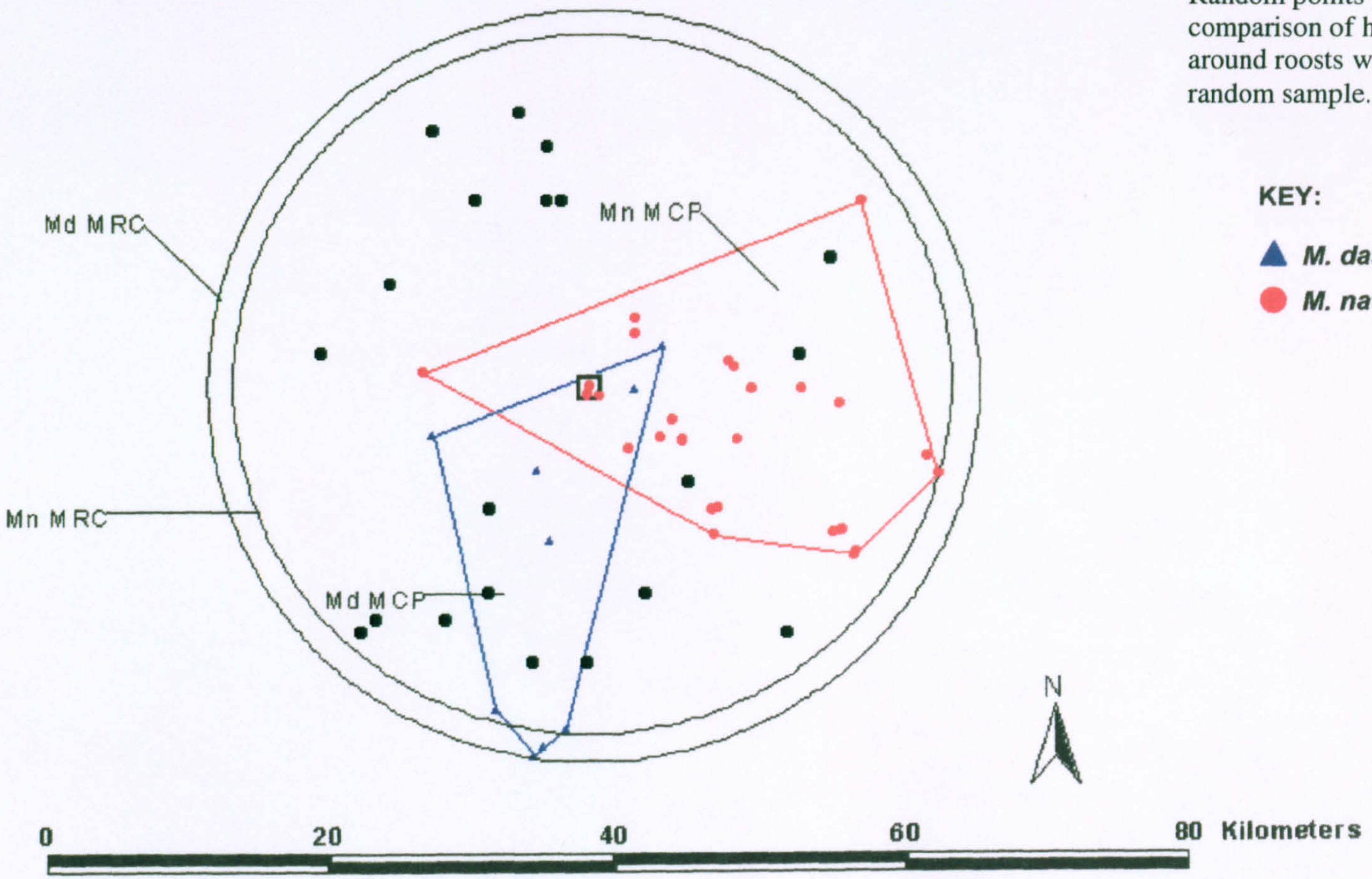


Fig. 5.1 overlay.
Random points used in comparison of habitats around roosts with a random sample.

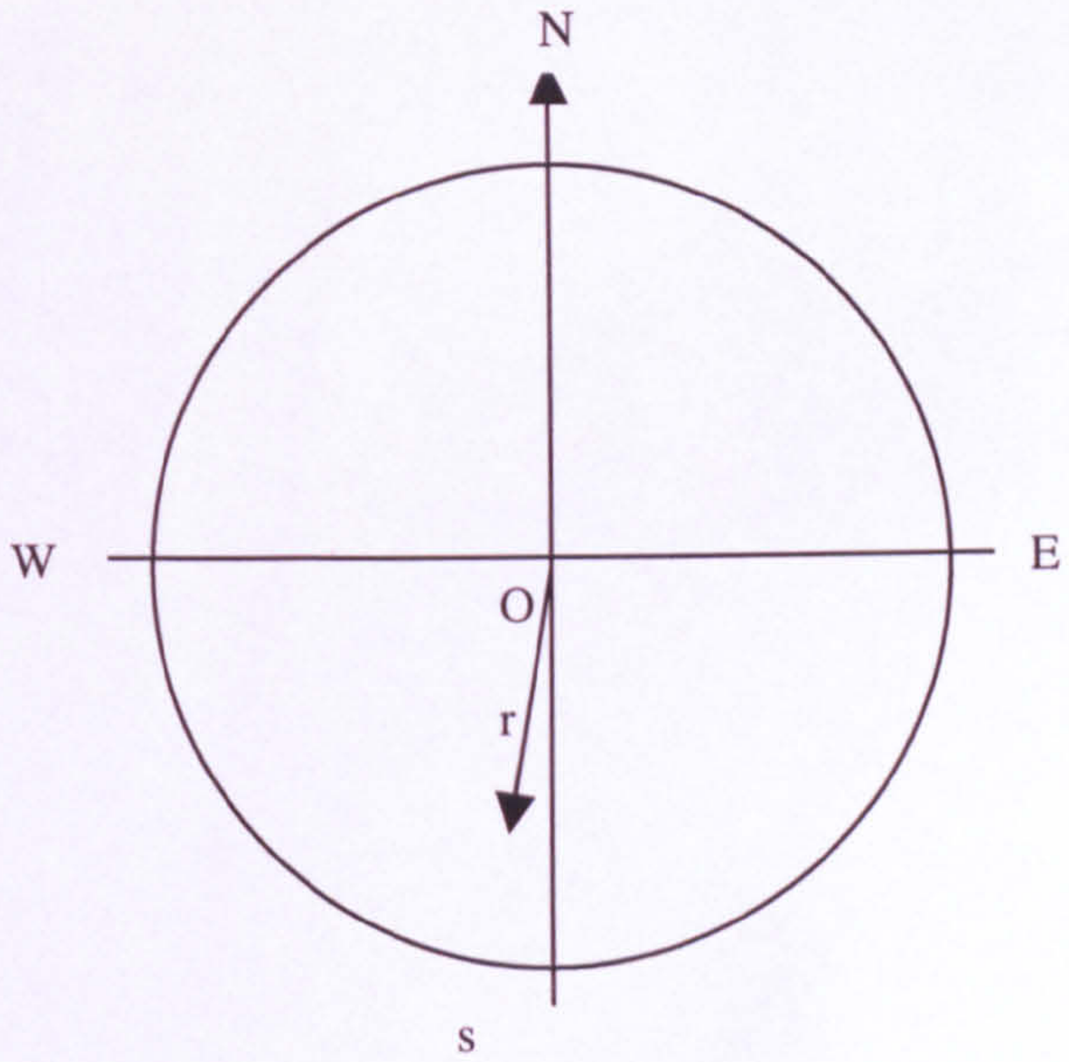
KEY:

▲ *M. daubentonii*

● *M. nattereri*

Figure 5.2a. Circular distribution of *M. daubentonii* day roosts around the origin

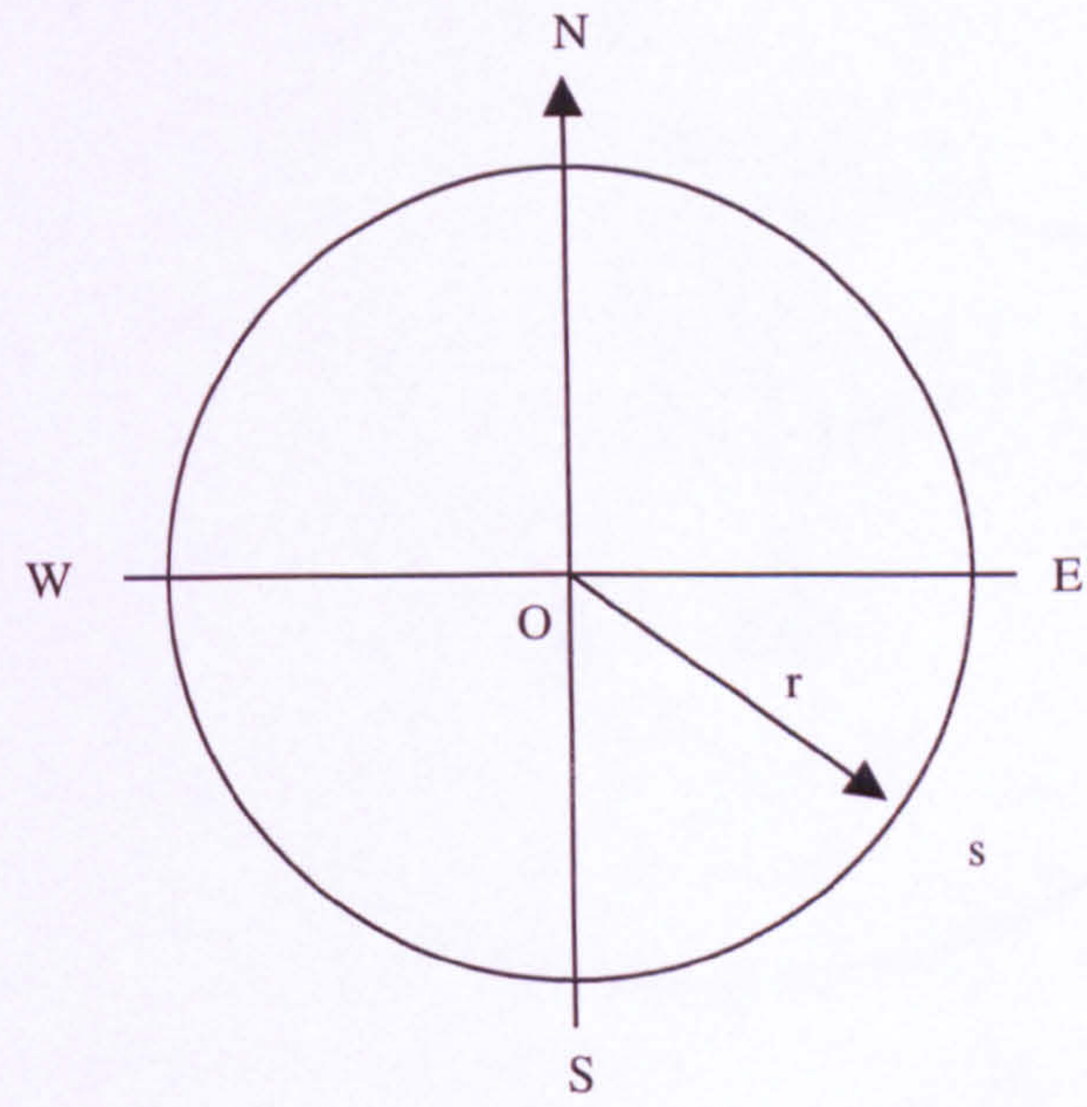
Goodness of fit Chi-square test for non-uniform distribution $_{(0.05,11)}$ = 35.896, P<0.001.
Significantly different from uniform.
Rayleigh's Test for non-random distribution R = 6.8713, P<0.001.
Significantly different from random.



r = 0.687
ā = 189°
s = 018°

Figure 5.2b. Circular distribution of *M. nattereri* day roosts about the origin

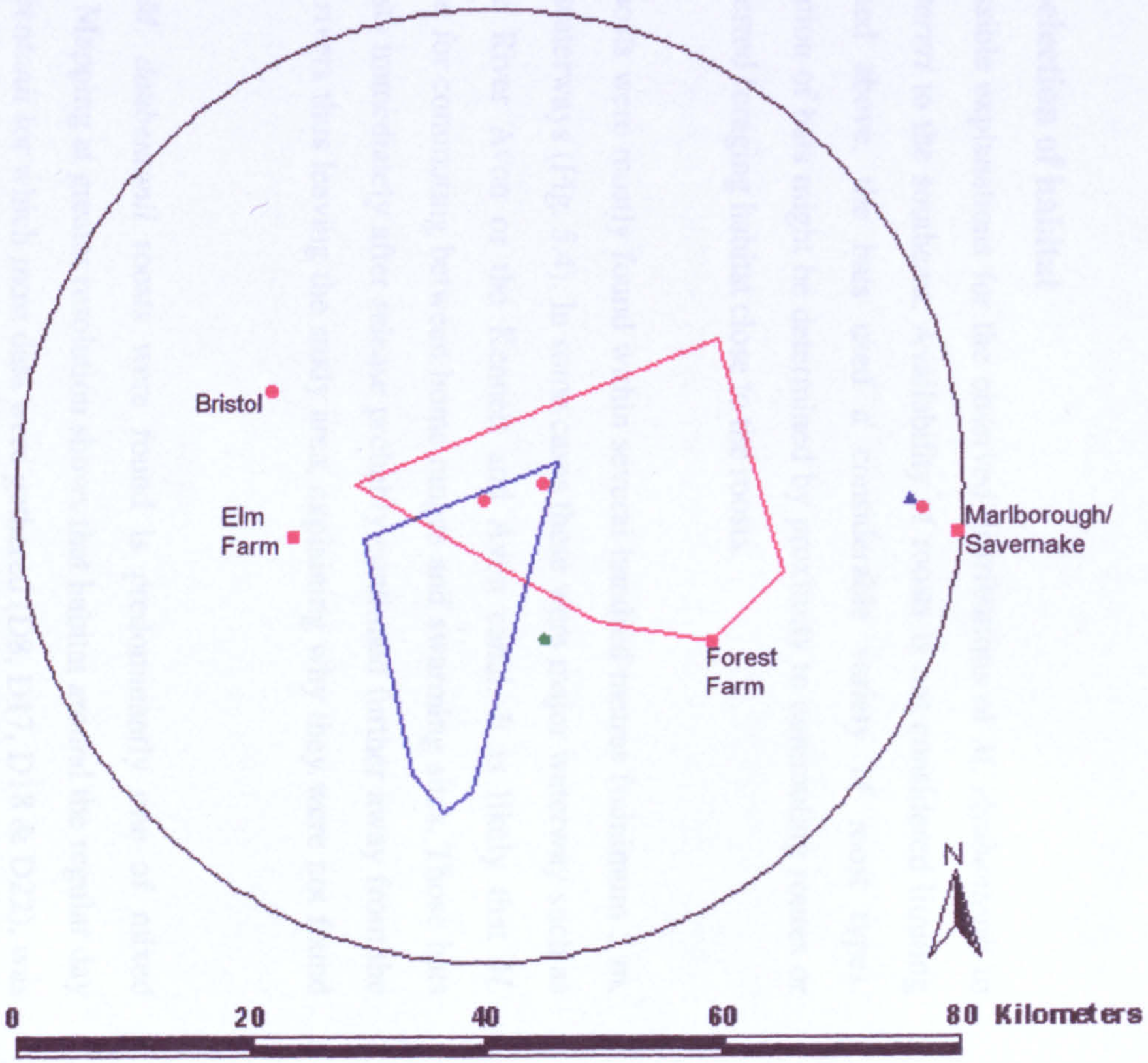
Goodness of fit Chi square test for non-uniform distribution $_{(0.05,21)}$ = 50.00, P<0.001.
Significantly difference from uniform.
Rayleigh's Test for non-random distribution R = 19.91, P<0.001.
Significantly different from random.



r = 0.905
ā = 128°
s = 005°

r = measure of concentration
(0 – not concentrated, to 1 - concentrated)
ā = mean angle
s = standard deviation

Figure 5.3. Map showing the location of ringed bats found away from the study site (Box) where they were ringed and the location of ringing of bats ringed elsewhere but caught at the swarming site. Minimum convex polygons (MCPs) constructed from day roosts of radio-tagged *M. nattereri* (Mn) and *M. daubentonii* (Md) are shown for comparison. A Maximum Range Circle (MRC) has been constructed from the radius of the furthest bat. Study site is at centre.



KEY:

● *M. nattereri* re-find away from the swarming site

■ Locations of ringing of three female *M. nattereri* captured later at the swarming site

▲ *M. daubentonii* re-find away from the swarming site

⬟ *M. bechsteinii* re-find away from the swarming site

5.3.4. Day roost types

M. daubentonii exclusively used trees, predominantly oak (*Quercus* spp., 86%, n = 7), as roosts, but not the oldest trees available in the parkland. *M. nattereri* used broad-leaved trees (54%) and a wide variety of buildings (46%). Tree species used included: white willow (*Salix alba*), oak (*Quercus* spp.), ash (*Fraxinus excelsior*), apple and common lime (*Tilia vulgaris*) (Plate 5.5). Buildings included wooden barns (n = 3), stone-built garages (n = 2), derelict (n = 1) and occupied dwellings (n = 3), a chapel (n = 1) and an icehouse (n = 1) (Plate 5.5). Sixteen emergence counts showed that the bats were generally roosting alone. *M. nattereri* with more than one roost used both buildings and trees, and males and females both used buildings and/or trees. Two female *M. nattereri* repeatedly roosted in the mine within 15m of the entrance. Several others spent the first day after release in the mine. The bats could not be seen and I realized just how difficult *Myotis* bats are to see in such places when they hide themselves in cracks and crevices in the walls and ceiling.

5.3.5. Large-scale selection of habitat

There are several possible explanations for the observed distributions of *M. daubentonii* to the south and *M. nattereri* to the southeast. Availability of roosts is not considered limiting because, as mentioned above, the bats used a considerable variety of roost types. Alternatively, distribution of bats might be determined by proximity to commuting routes or by availability of preferred foraging habitat close to the roosts.

All *M. daubentonii* roosts were mostly found within several hundred metres (minimum 5 m, maximum 400 m) of waterways (Fig. 5.4). In some cases these were major waterway such as the River Frome, the River Avon or the Kennett and Avon canal. It is likely that *M. daubentonii* used these for commuting between home ranges and swarming sites. Those bats using transitional roosts immediately after release probably continued further away from the release site along the rivers thus leaving the study area, explaining why they were not found again.

The area in which *M. daubentonii* roosts were found is predominantly one of mixed agriculture (Fig. 5.5). Mapping at greater resolution shows that habitat around the regular day roosts of four *M. daubentonii* for which most data were gathered (D8, D17, D18 & D22), was dominated by parkland, woodland and water habitats (Fig. 5.6, Plate 5.6). Bat D4 used a tree overhanging the River Avon for only one night before moving on. The habitat around this presumed transitional roost is in marked contrast to that around the roosts of the other four bats depicted (Fig. 5.6). The sample size is not great enough for statistical analysis comparing habitat around roosts of *M. daubentonii* with that around random points. However roosts

were situated in areas richer in Parkland, Open Water and Woodland habitat than the random sample (Fig. 5.7). The habitat around the transitional roost had a very different composition because of its proximity to a large urban area.

The MCP for *M. nattereri* roosts is also in an area of mixed agriculture, between two predominantly arable areas (Fig. 5.5). Habitat within 2 km of the day roosts of *M. nattereri* was more varied than for *M. daubentonii* (Fig. 5.8) and by visual inspection did not appear to differ markedly from the random points (Fig. 5.9). However, habitat around roosts was significantly different from the random sample ($\chi^2 = 37.15$, d.f. = 7, $P<0.0001$) meaning that habitat was not found around roosts at random. The compositional analysis produced a ranking matrix (Table 5.1), which can be summarized from habitat most found around roosts to least found as follows:

Arable > Pasture > Parkland > Urban > Woodland > Open Water > Amenity >>> Scrub

(where the symbol '>' denotes a non-significant preference for the habitat type preceding the symbol over the habitat type immediately following the symbol (e.g. between Arable and Pasture above) and '>>>' denotes a significant preference of the habitat type preceding over the habitat type following the symbol (for example between Amenity and Scrub above)). From Table 5.1 it is seen that, although there is no significant difference between Arable and Pasture, nor between Pasture and Parkland, there is a significant difference between Arable and all other habitats, except Open Water (and Pasture) and between Pasture and all other habitats, except Open Water (and Arable). Scrub was found significantly less around roosts compared to other habitat types than expected from the random values, however the mean percentage cover of Scrub in the random sample is greatly inflated by one random point that was on the heathland of Salisbury Plain in the south-east of the study area. If this one point is eliminated from the analysis the ranked order remains the same but Scrub is no longer statistically different from all other habitat types.

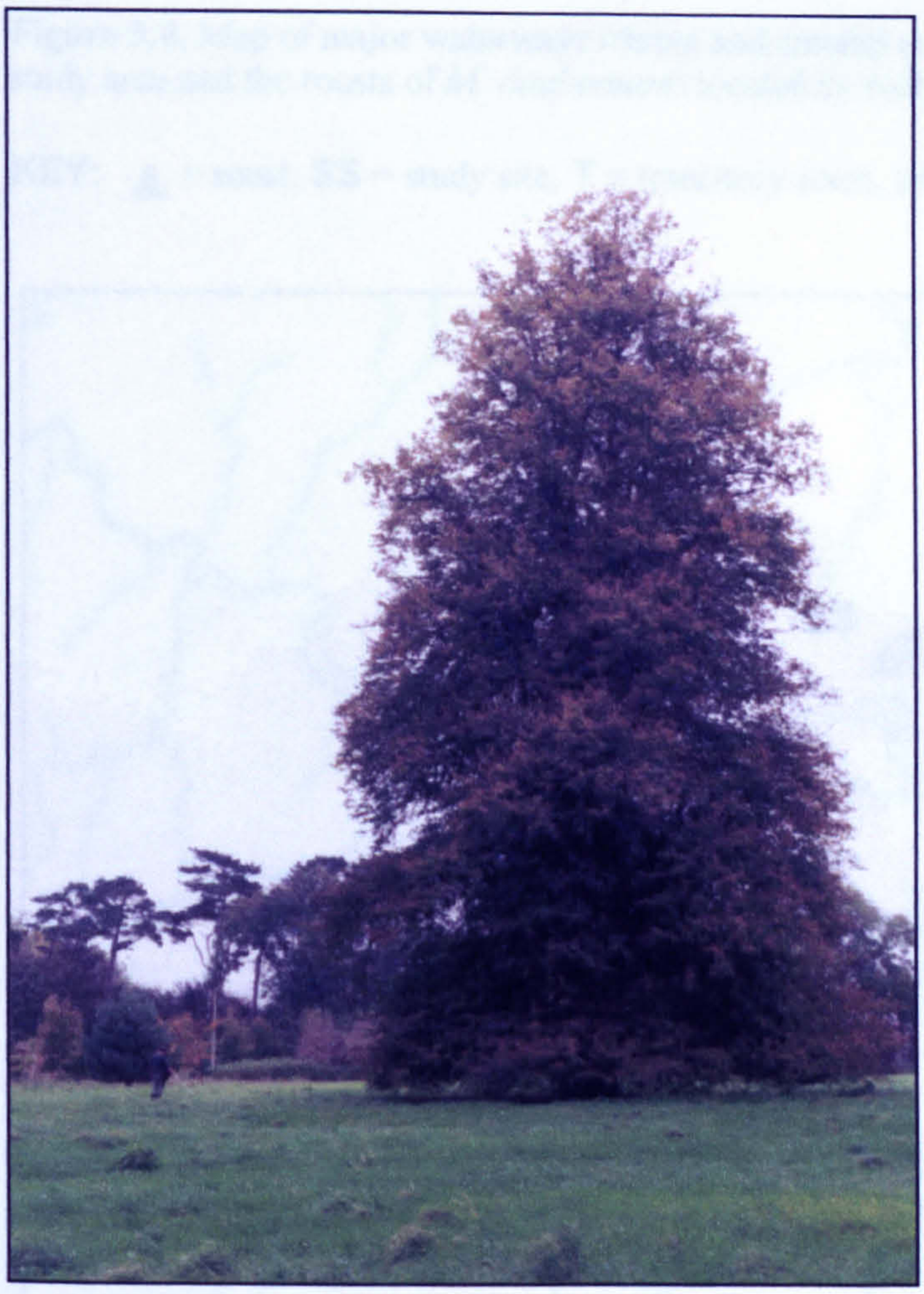


Plate 5.5.
Examples of day roosts of *M. nattereri*

Top – a lime (linden) tree

Bottom – an ice-house

Figure 5.4. Map of major waterways (rivers and canals) within the southern region of the study area and the roosts of *M. daubentonii* located by radio-tracking.

KEY: ▲ = roost, **SS** = study site, **T** = transitory roost, **pl** = found by plane only

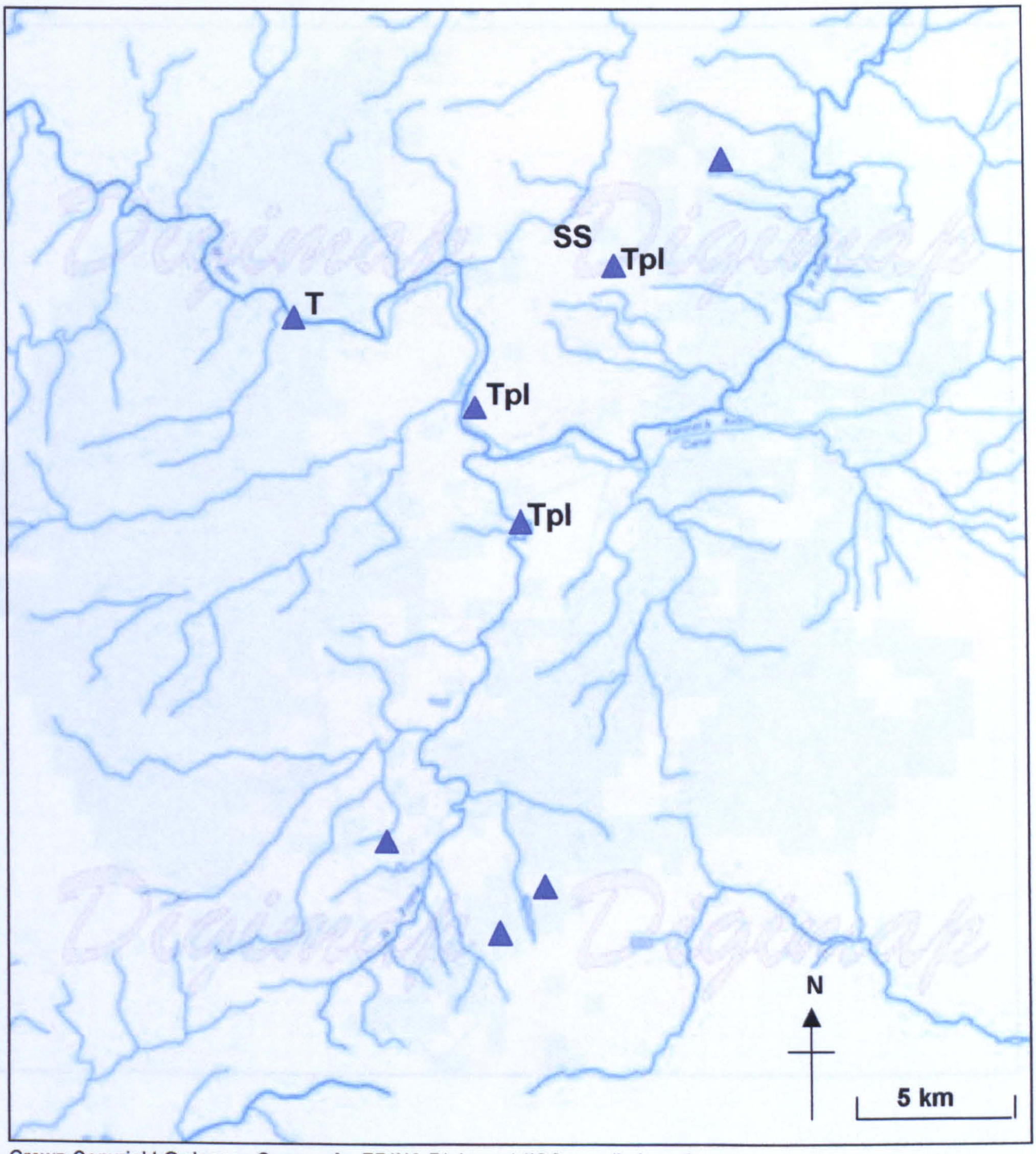


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Figure 5.5. Land classification for 1 km squares as arable or pastural according to the land class database held by CEH (Bunce *et al*. 1996) with Minimum Convex Polygons (MCPs) for day roosts of radio-tagged *M. daubentonii* (Md) and *M. nattereri* (Mn).

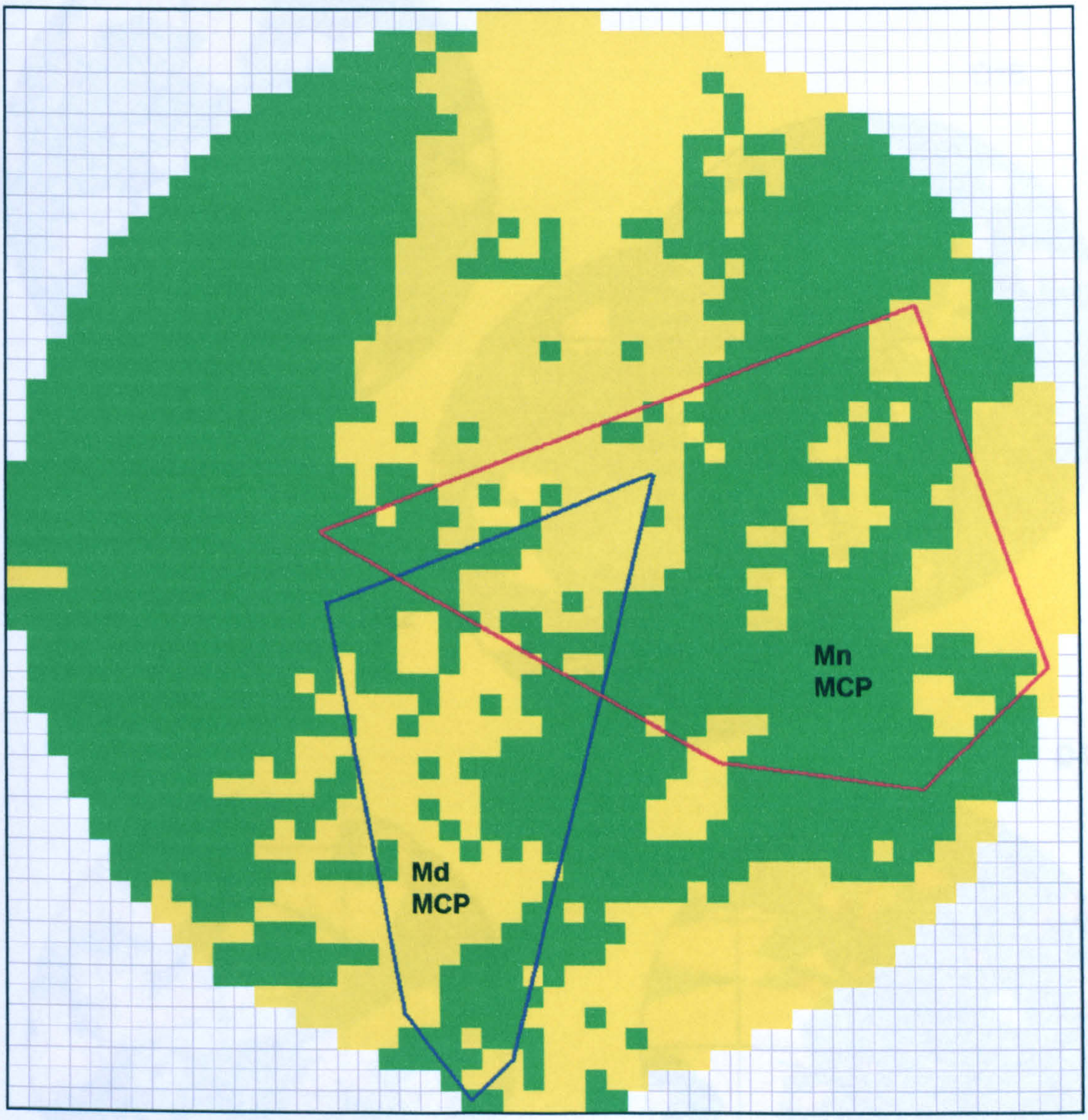
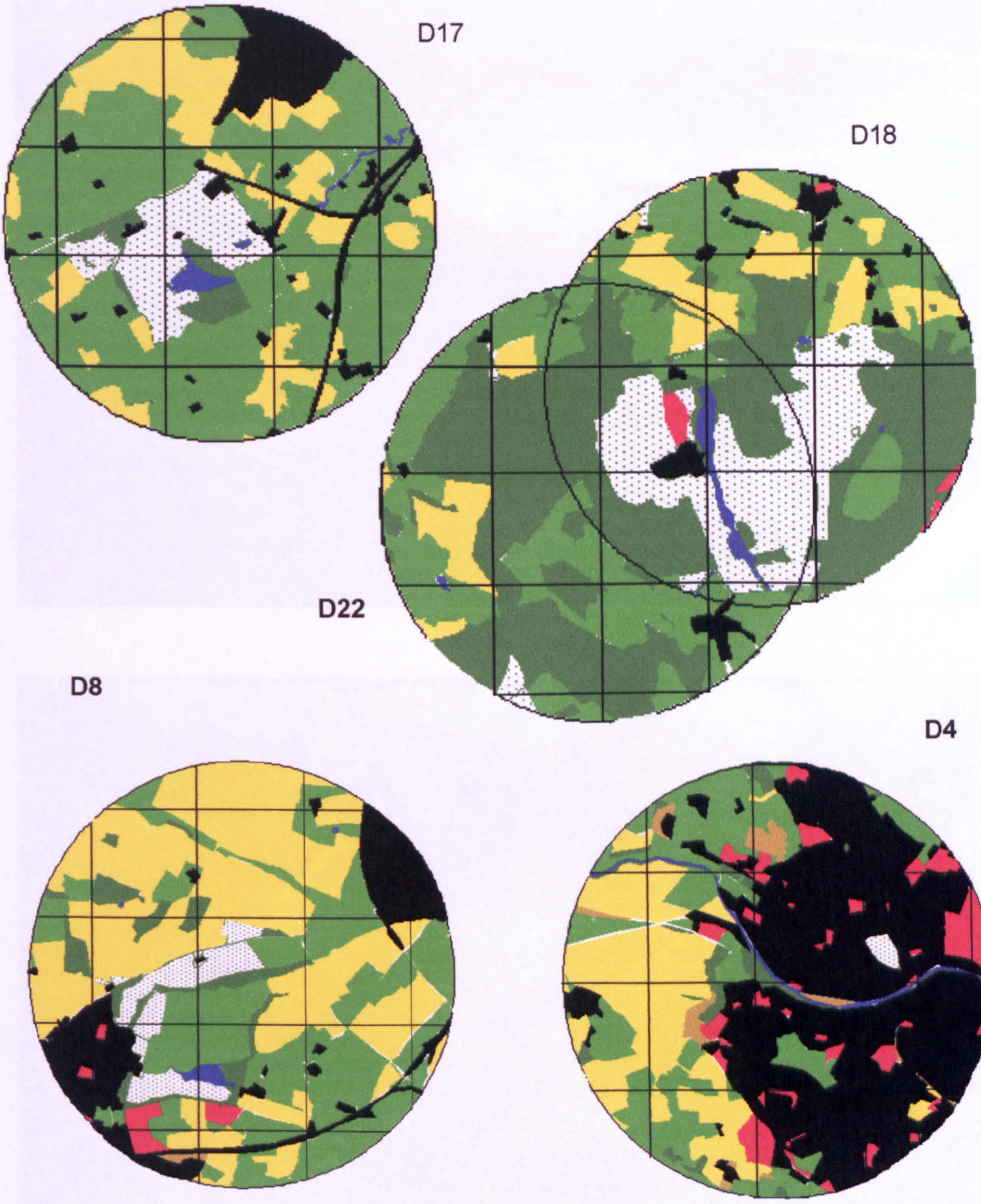


Figure 5.6. Habitat within 2 km of *M. daubentonii* roosts. Bats D8, D17, D18 and D22 demonstrated high fidelity to their day roosts and used habitat within these areas for feeding. D4 moved from its area after only one day.



KEY:
Amenity Arable Parkland Pasture Open Water Scrub Urban Woodland
Each square measures 1 x 1 km. Roost is at center of circle.

Plate 5.6. Habitat used by *M. daubentonii* and *M. nattereri*.

Top - Corsham Court estate, including Mynte Wood (where bat D8 day roosted) and Corsham Lake (where D8 predominantly foraged).
Bottom - Lackham Agricultural College and the River Avon (where bat N2 roosted and foraged.

Figure 5.7. Mean (+SE) percentage cover of each habitat around roosts (D8, D17, D18, D22), around random points and around the transitory roost (D4) for *M. daubentonii*

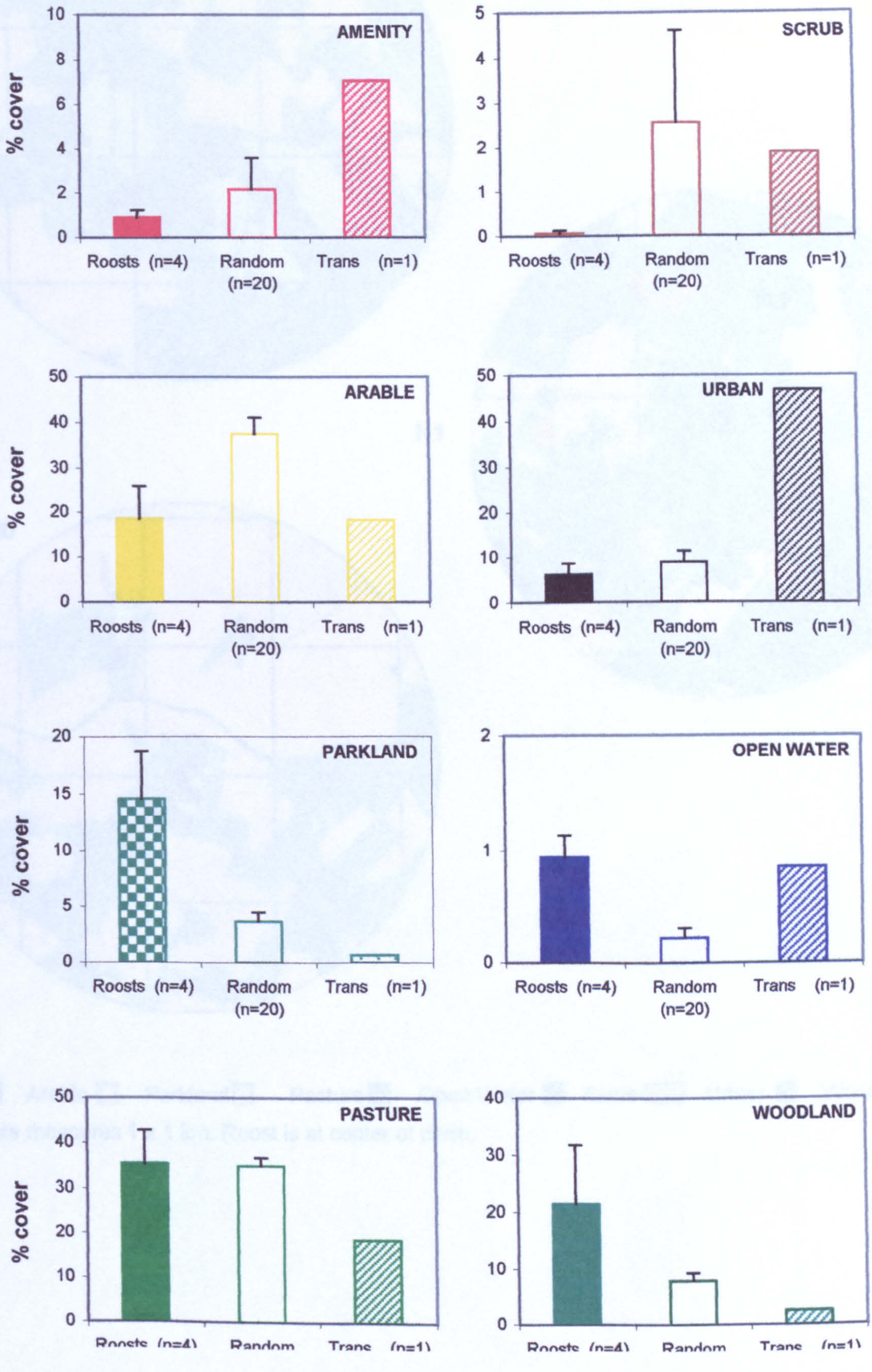


Figure 5.8. Examples of habitat composition within 2 km of *M. nattereri* roosts.

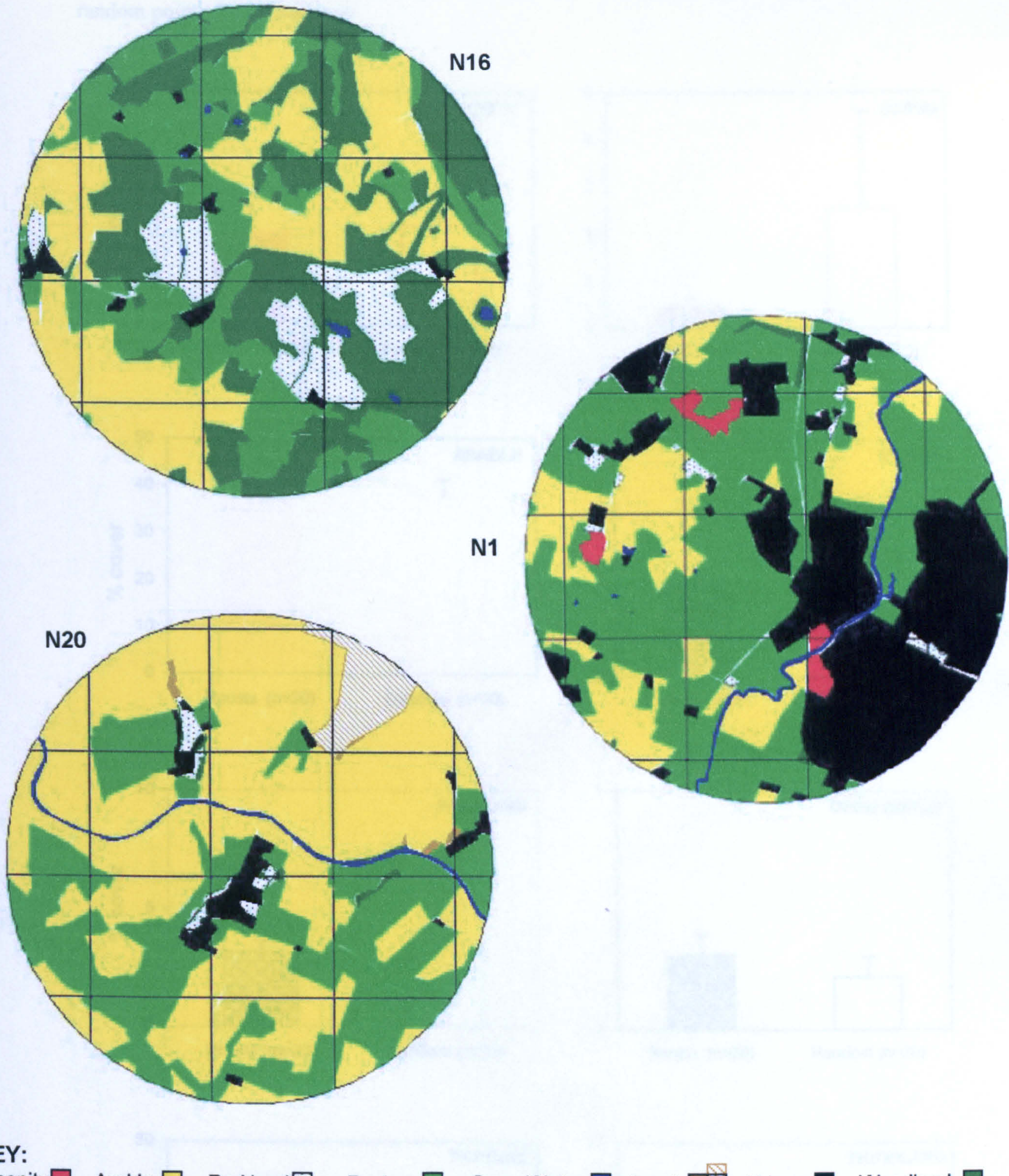


Figure 5.9. Mean (+SE) percentage cover of each habitat around roosts and around random points for *M. nattereri*

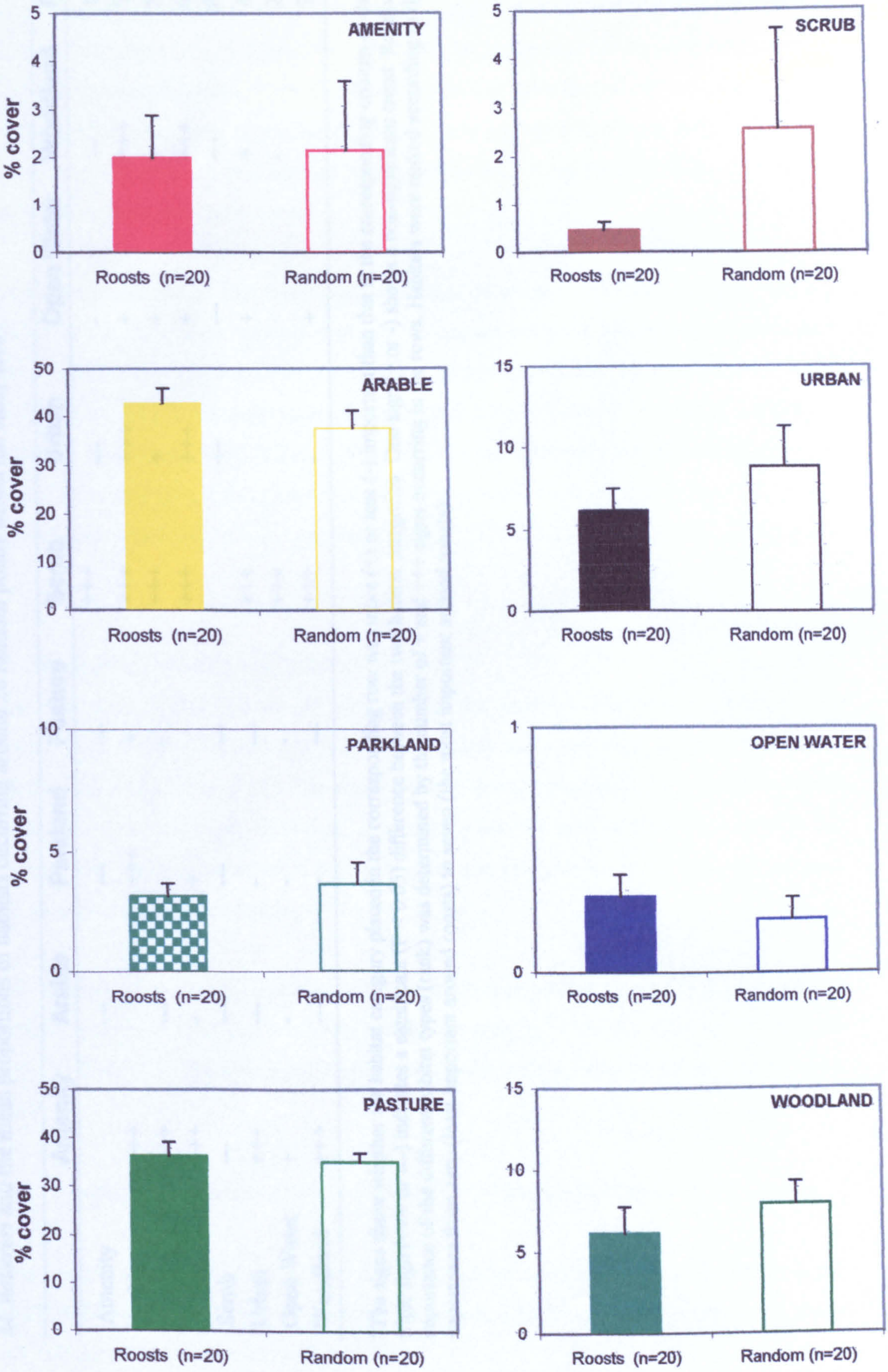


Table 5.1. Ranking matrix for *M. nattereri* based on comparing proportions of habitats occurring around roosts of 20 individual *M. nattereri* and the mean proportions of habitats occurring around 20 random points within the study area[a].

	Amenity	Arable	Parkland	Pasture	Scrub	Urban	Open Water	Woodland	Rank
Amenity		---	---	---	+++	---	-	---	1
Arable	+++		+++	+	+++	+++	+	+++	7
Parkland	+++	---		-	+++	+	+	+	5
Pasture	+++	-	+		+++	+++	+	+++	6
Scrub	---	---	---	---		---	---	---	0
Urban	+++	---	-	---	+++		+	+	4
Open Water	+	-	-	-	+++	-		-	2
Woodland	+++	---	-	---	+++	-	+		3

[a] The signs show whether the habitat category placed in the corresponding row was more (+) or less (-) important than that in the corresponding column of the matrix. A triple sign (+++ or ---) indicates a significant ($P < 0.05$) difference between the two habitat categories. One sign (+ or -) shows a non-significant trend. Relative importance of the different habitat types (rank) was determined by the number of + and +++ signs occurring in the rows. Habitats were ranked according to their importance from zero (least important around roosts) to seven (the most important around roosts).

5.3.6. Home range parameters

Local home ranges were constructed for four *M. daubentonii* and 13 *M. nattereri* where home range reached or approached an asymptote. Asymptotes were normally reached after approximately 30 fixes collected over at least two nights of tracking (Fig. 5.10). Examples of home range plots are given in Figure 5.11. Home range parameters can be compared between species and between the sexes (Table 5.2). Core areas represent foci of activity; for the most part these are foraging areas. Occasionally, males spent a great deal of time in the vicinity of their roosts while active. It was unclear whether they were foraging or perhaps involved in defending their roost from rival males.

Ten bats had home ranges that overlapped with that of at least one other bat, but with the exception of one pair there was no evidence of contact between individuals. A male and female *M. daubentonii* (D18 & D22) were on one occasion in exactly the same location at the same time (Fig. 5.11b), although their coincidence at a roost used by D18 lasted for only a few minutes. For example bats N10 and N11 lived in close proximity but foraged on opposite sides of a main road (Fig. 5.11c), bats N18 and N19 roosted in and foraged in the same valley but in different areas (Fig. 5.11d) and bats D22 and D17 fed on different parts of the same lake.

5.3.7. Small-scale selection of habitat

Sample size was insufficient for *M. daubentonii* for analysis. However a trend toward Amenity, Open Water, Parkland and Woodland habitats and away from Arable, Pasture and Urban habitats is seen (Fig. 5.12). The trend toward Amenity land might sound incongruous, however the home ranges of the two *M. daubentonii* that foraged on Half-Mile pond in the Longleat Estate included sections of a caravan park and playground on the edge of the pond which account for this observation.

For *M. nattereri* there was a significant difference between habitat found in the home ranges (80% UD) compared with that available around the roost ($\chi^2 = 16.52$, d.f.= 6, $P = 0.0112$) (Fig. 5.13, Table 5.3). Habitat types ranked from most to least used on home ranges are as follows:

Woodland > Pasture > Arable > Open Water > Urban > Parkland > Amenity

Habitat composition in core areas (50% UD) did not differ significantly from that in the home range (80% UD) ($\chi^2 = 4.88$, d.f.= 5, P =0.43). However, a preference towards Parkland, Open Water and Woodland can be seen (Fig. 5.13).

Figure 5.10. Examples of asymptote plots for radio-tracked bats showing how MCP area increased with the number of fixes obtained.

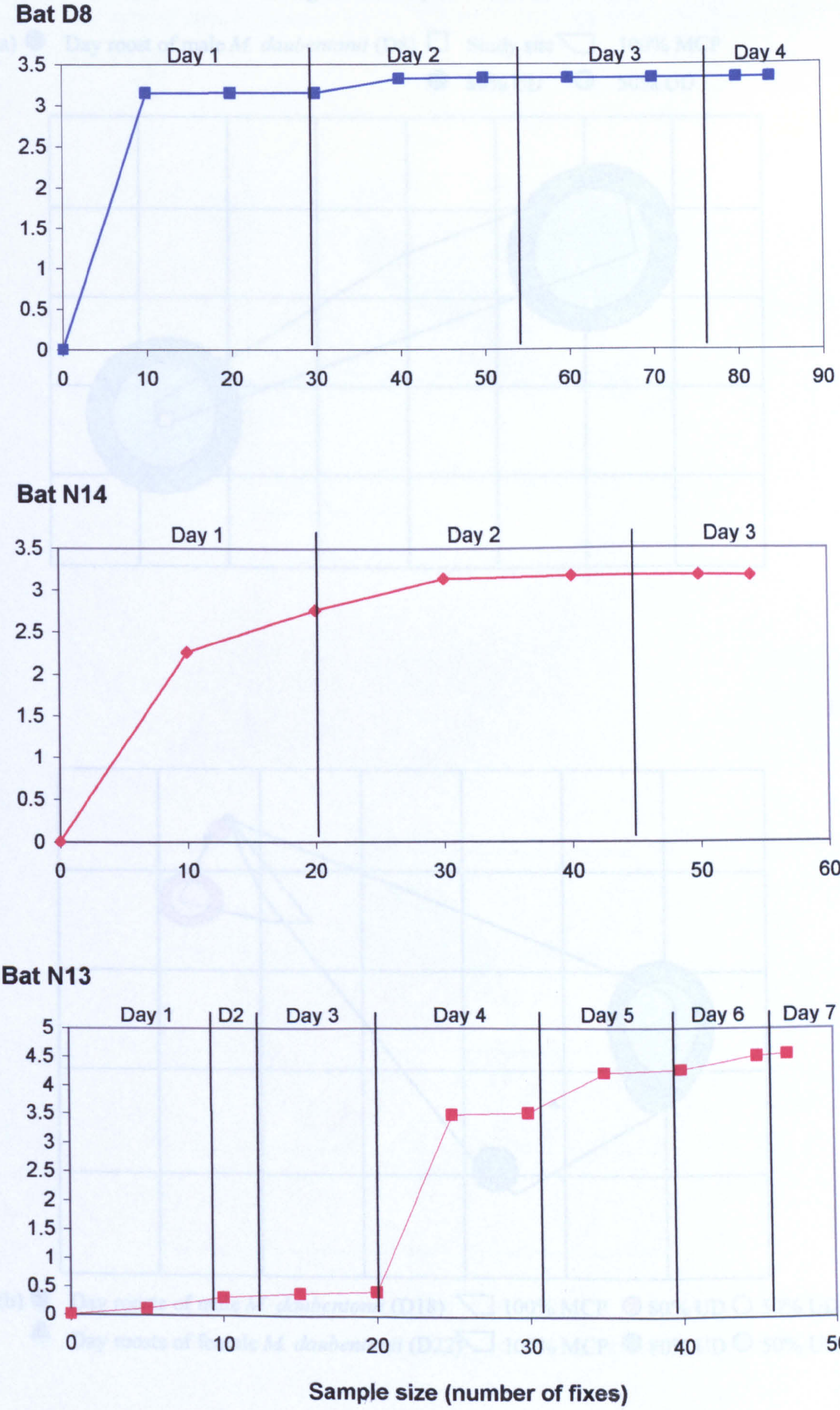
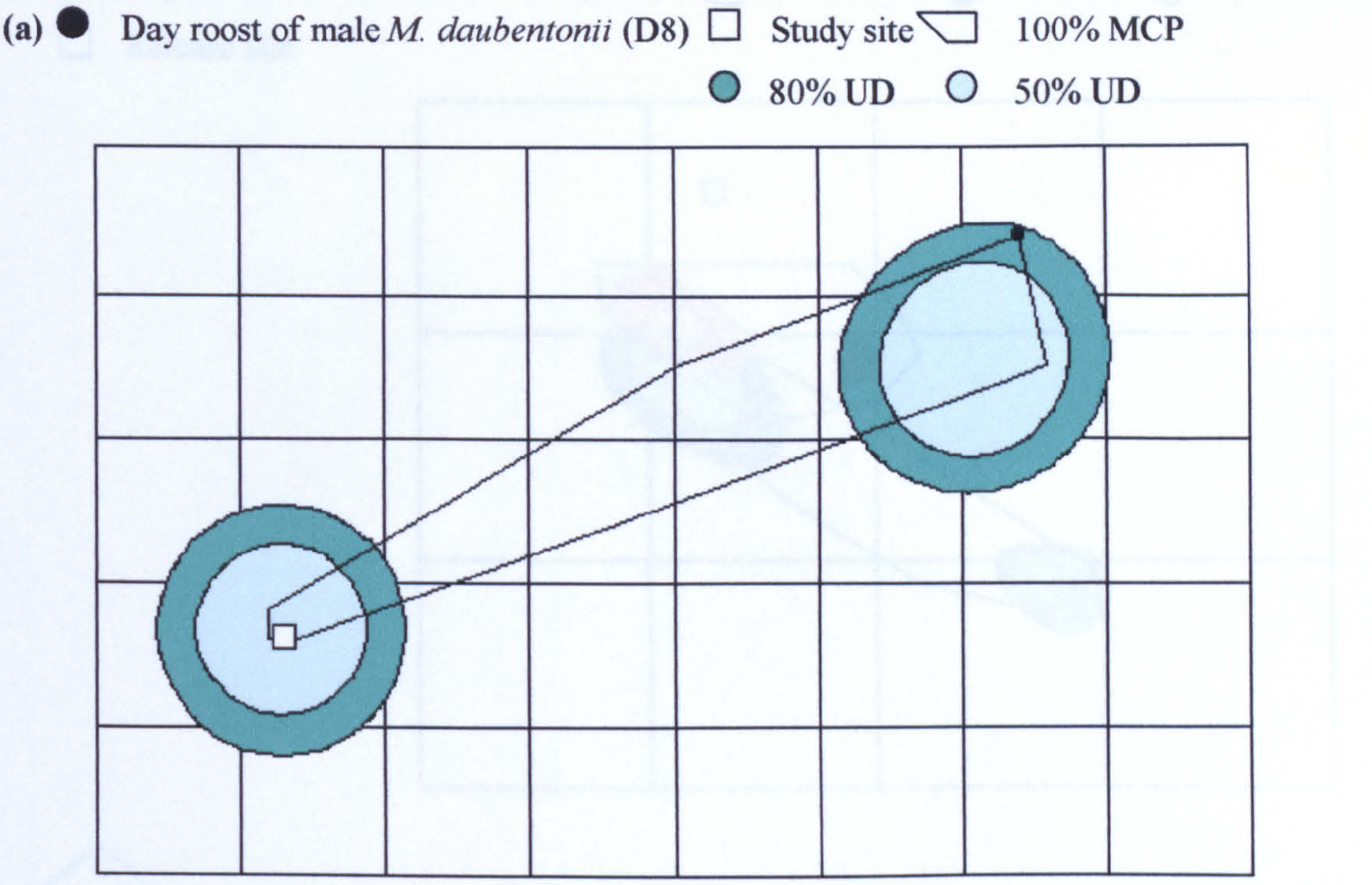


Figure 5.11. Examples of home range plots for radio-tracked bats, showing 100% minimum convex polygons, 80% and 50% utilization distributions. MCPs and UDs were calculated from fixes collected over several nights. Each square measures 1 x 1 km.



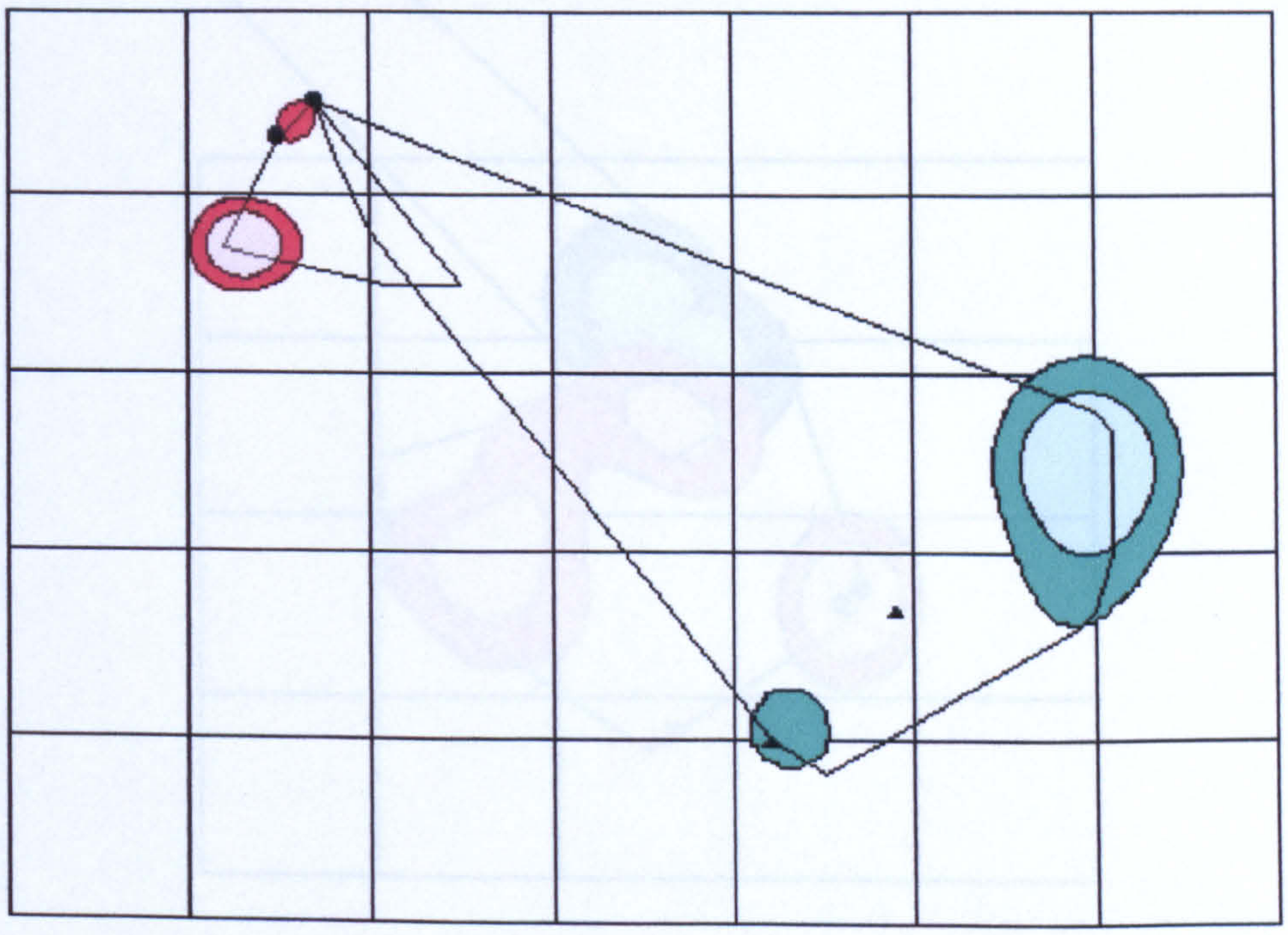
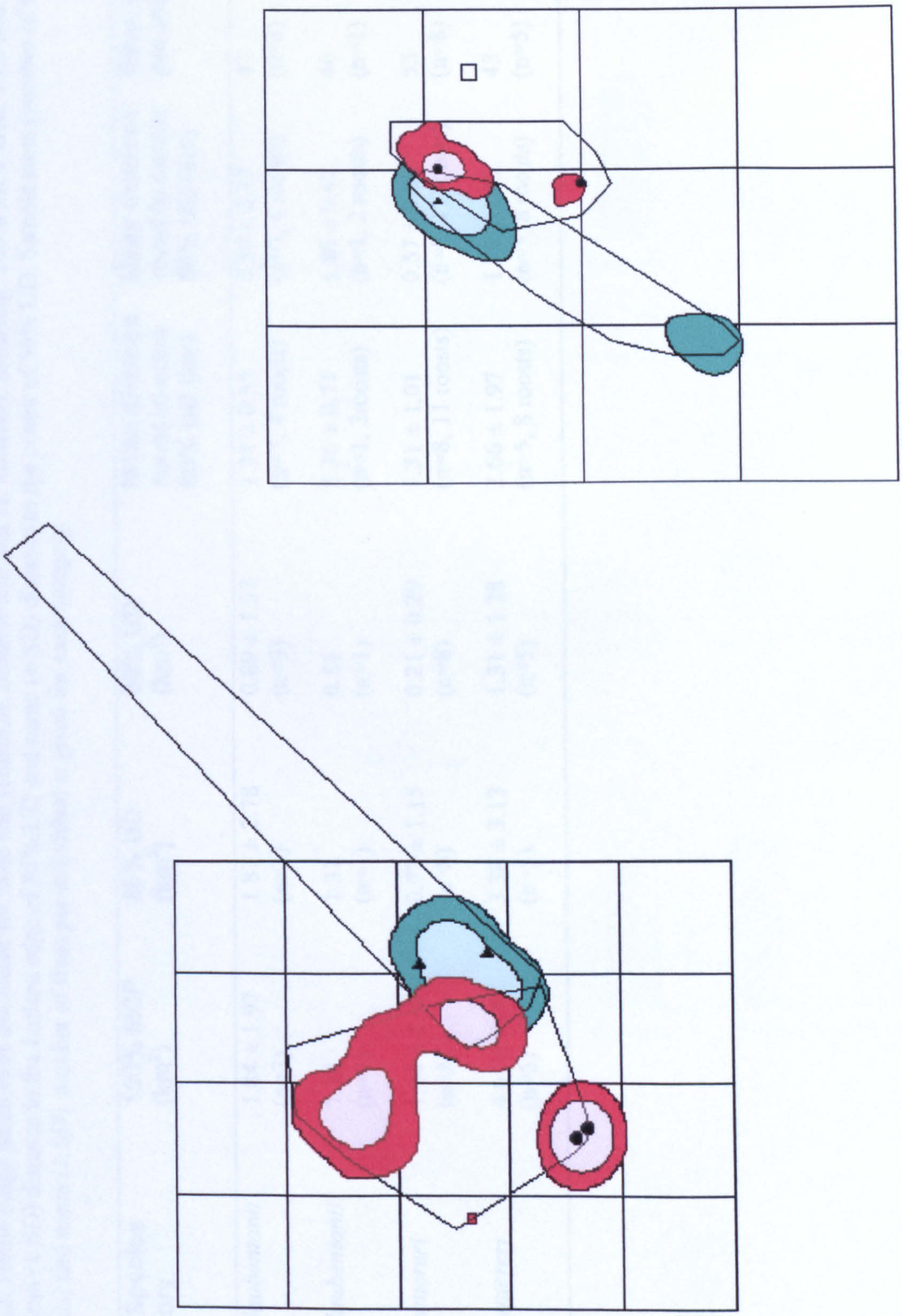


Figure 5.11. cont.

(c) ● Day roosts of male *M. nattereri* (N10) 100% MCP 80% UD 50% UD

▲ Day roost of male *M. nattereri* (N11) 100% MCP 80% UD 50% UD

□ Release site



(d) ● Day roosts of female *M. nattereri* (N18) 100% MCP 80% UD 50% UD

▲ Day roosts of female *M. nattereri* (N19) 100% MCP 80% UD 50% UD

Table 5.2. Home range parameters calculated for male and female *M. daubentonii* and *M. nattereri*, including: 100% MCP area, 80% and 50% UD areas, mean (± SD) distance to the furthest edge of 80% UD and mean (± SD) distance to the center of 50% UD. Sample sizes (number of individuals and roosts) and mean (± SD) number of fixes per individual is given for each category.

Sex / Species category	100% MCP (km^2)	80% UD (km^2)	50% UD (km^2)	Mean distance roost to edge 80% UD (km)	Mean distance roost to center 50% UD (km)	Mean n. fixes per individual
♂ *M. daubentonii*	1.84 ± 1.97 (n=3)	1.87 ± 2.78 (n=3)	0.89 ± 1.37 (n=3)	1.24 ± 0.55 (n=3, 4 roosts)	0.59 ± 0.37 (n=3, 4 roosts)	47 (n=3)
♀ *M. daubentonii*	7.16 (n=1)	1.31 (n=1)	0.51 (n=1)	2.36 ± 0.71 (n=1, 2roosts)	1.86 ± 0.67 (n=1, 2 roosts)	46 (n=1)
♂ *M. nattereri*	1.64 ± 1.42 (n=8)	0.77 ± 1.15 (n=8)	0.21 ± 0.29 (n=8)	1.31 ± 1.01 (n=8, 11 roosts)	0.57 ± 0.89 (n=8, 11 roosts)	53 (n=8)
♀ *M. nattereri*	4.67 ± 2.90 (n=5)	3.38 ± 3.13 (n=5)	1.31 ± 1.28 (n=5)	2.66 ± 1.97 (n=5, 8 roosts)	1.76 ± 1.49 (n=5, 8 roosts)	43 (n=5)

Figure 5.12. Mean (+SE) percentage cover of each habitat 'available' (2 km around roosts) compared to that 'used' 80% and 50% of the time by *M. daubentonii* (n=4).

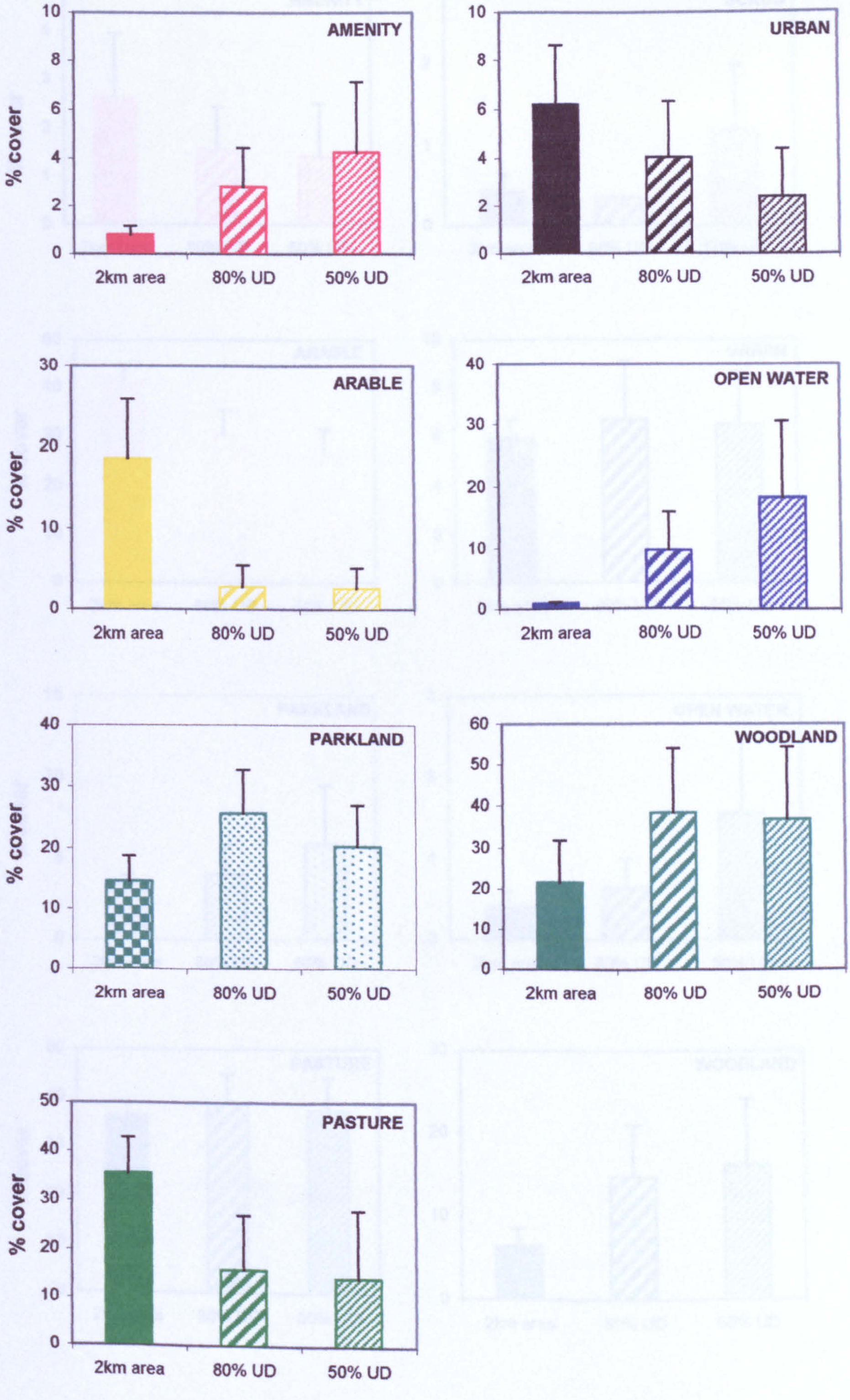


Figure 5.13. Mean (+SE) percentage cover of each habitat 'available' (2 km around roosts) compared to that 'used' 80% and 50% of the time by *M. nattereri* (n=13)

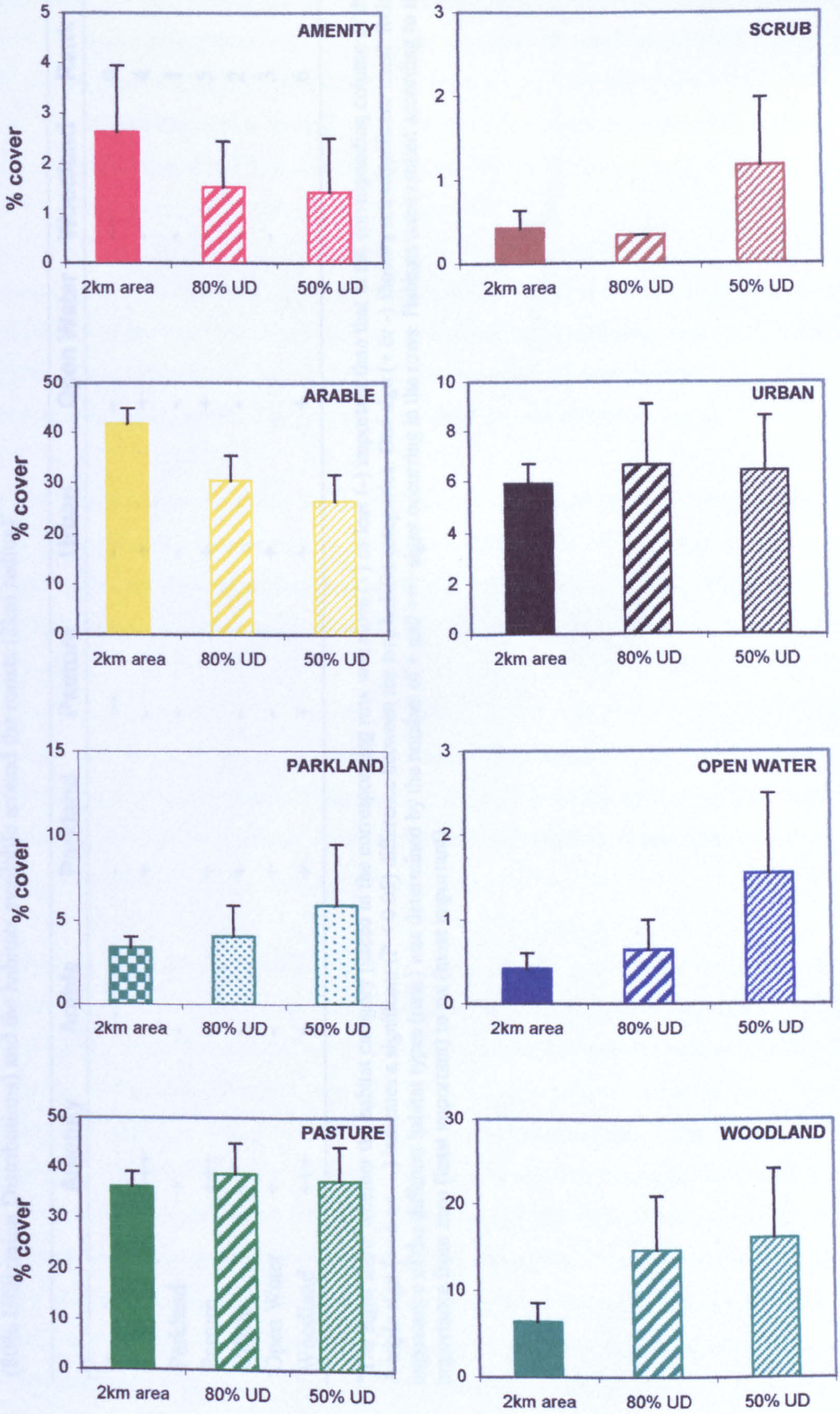


Table 5.3. Ranking matrix for *M. nattereri* based on comparing proportions of habitats occurring within individual home ranges (80% Utilization Distributions) and the habitats available around the roosts (2km radius)[a].

	Amenity	Arable	Parkland	Pasture	Urban	Open Water	Woodland	Rank
Amenity		---	-	---	-	-	---	0
Arable	+++		+	-	+	+	-	4
Parkland	+	-		-	-	-	-	1
Pasture	+++	+	+		+	+	-	5
Urban	+	-	+	-		-	-	2
Open Water	+	-	+	-	+		-	3
Woodland	+++	+	+	+	+	+		6

[a] The signs show whether the habitat category placed in the corresponding row was more (+) or less (-) important than that in the corresponding column of the matrix. A triple sign (+++ or ---) indicates a significant ($P < 0.05$) difference between the two habitat categories. One sign (+ or -) shows a non-significant trend. Relative importance of the different habitat types (rank) was determined by the number of + and +++ signs occurring in the rows. Habitats were ranked according to their importance from zero (least important) to six (most important).

5.3.8. Nightly activity budgets

On average, bats left their roosts 85 minutes after official sunset however there was considerable variation among and within individuals (min 38, max 147 minutes after sunset) (Fig. 5.14). Mean emergence times (± SD) post-sunset for *M. daubentonii* (76 ± 33 minutes, n=9) and *M. nattereri* (88 ± 22 minutes, n=30) were not significantly different (t = -1.03, d.f. = 10, P = 0.33). There was no difference between the sexes in emergence time for *M. daubentonii* (t = 0.11, d.f. = 5, P = 0.92) but there was for *M. nattereri* (t = 2.39, d.f. = 14, P = 0.031). Males emerged later on average than females.

Two *M. daubentonii* spent the first day after release in the mine and although these data are not included in the analyses above they are of interest because the bats emerged much later from the mine (147 and 105 minutes after sunset respectively) than from their tree roosts (57 and 68 minutes after sunset). The prevailing weather was similar on both days.

Time of return to the roost was highly variable for individual bats and among individuals (from 160 minutes before sunrise to 54 minutes after sunrise) (Fig. 5.15). *M. daubentonii* returned with less time remaining before sunrise than *M. nattereri* (t = 7.85, d.f = 21, P <0.0001) however the records for *M. daubentonii* are for earlier in the year when the nights were shorter.

Most bats were active for at least six hours per night. On average they were active for 55 ± 22% of the time between sunset and sunrise. Increasingly from the start of October onwards bats were inactive for long periods of time in the middle of the night, and often would only feed for a short time after dusk and not emerge again.

Nightly activity budgets of the bats therefore varied according to time of year, but there was also variation between individuals in their activity. Most *M. daubentonii* foraged immediately after emerging from the roost and sometimes again before dawn with a period of inactivity in the middle of the night. However, one (D8 – Fig. 5.11a) regularly visited the study site after foraging and therefore has a very different nocturnal time budget from the others. After emerging it foraged for between 56 and 142 minutes before commuting to the release site. On three consecutive evenings the bat spent between 145 and 183 minutes in the immediate vicinity of the mine entrance. 96% of the time it was either stationary or active in the tree canopy without moving very far. It ventured underground for only short durations (5 to 10 minutes) and then returned to the same foraging area and day roost. This bat roosted closer (5.8 km compared with 24.4, 25 and 26.7 km) to the mine than the other *M. daubentonii* that were not observed to return.

The shortest continuous bout of foraging activity by *M. daubentonii* was 50 minutes in duration and the longest 291 minutes. The female was most consistent in length of first foraging bout (230, 233 and 245 minutes on separate evenings in the same locality). The commuting time for the bats that remained on their home ranges was negligible. *M. daubentonii* tended to fly continuously when foraging, making occasional stops of short duration. One male remained active for 91% of the time between emergence and return.

Male *M. nattereri* spent a large proportion of time near to their roosts during the night, but were apparently active. Part of this activity may have constituted foraging, but perhaps they were guarding their roosts or females at the roost. During the night most *M. nattereri* showed repetitive behaviour, for example bats N10 and N11 (Fig. 5.11c) each made up to four short journeys away from the main roosts and feeding area to visit another roost or night roost and back again. The order in which bats visited foraging patches was consistent over the nights of observation.

5.3.9. Commuting flight speed

Most bats did not travel far between day roosts and foraging areas hence the length of commuting flights have not been calculated for all individuals. Two individuals made clear commuting movements when both start and end points could be determined. A female *M. daubentonii* flew directly from release to a roost at a speed calculated as 9.67 km/hr. A female *M. nattereri* roosting at the study site commuted directly on emergence to a feeding site 5.24 km away at an average flight speed of 14.5 ± 2.5 km/hr. On each occasion that bat D8 commuted between its foraging area and the study site it reached its destination more rapidly than the observers. It is assumed that it commuted straight to the site without foraging or diverting greatly from the straight-line course; therefore it must have traveled faster than 7.2 km/hr.

Figure 5.14. Emergence time of *M. daubentonii* and *M. nattereri* relative to time of sunset shown by the heavy line between 15 August and 31 October.

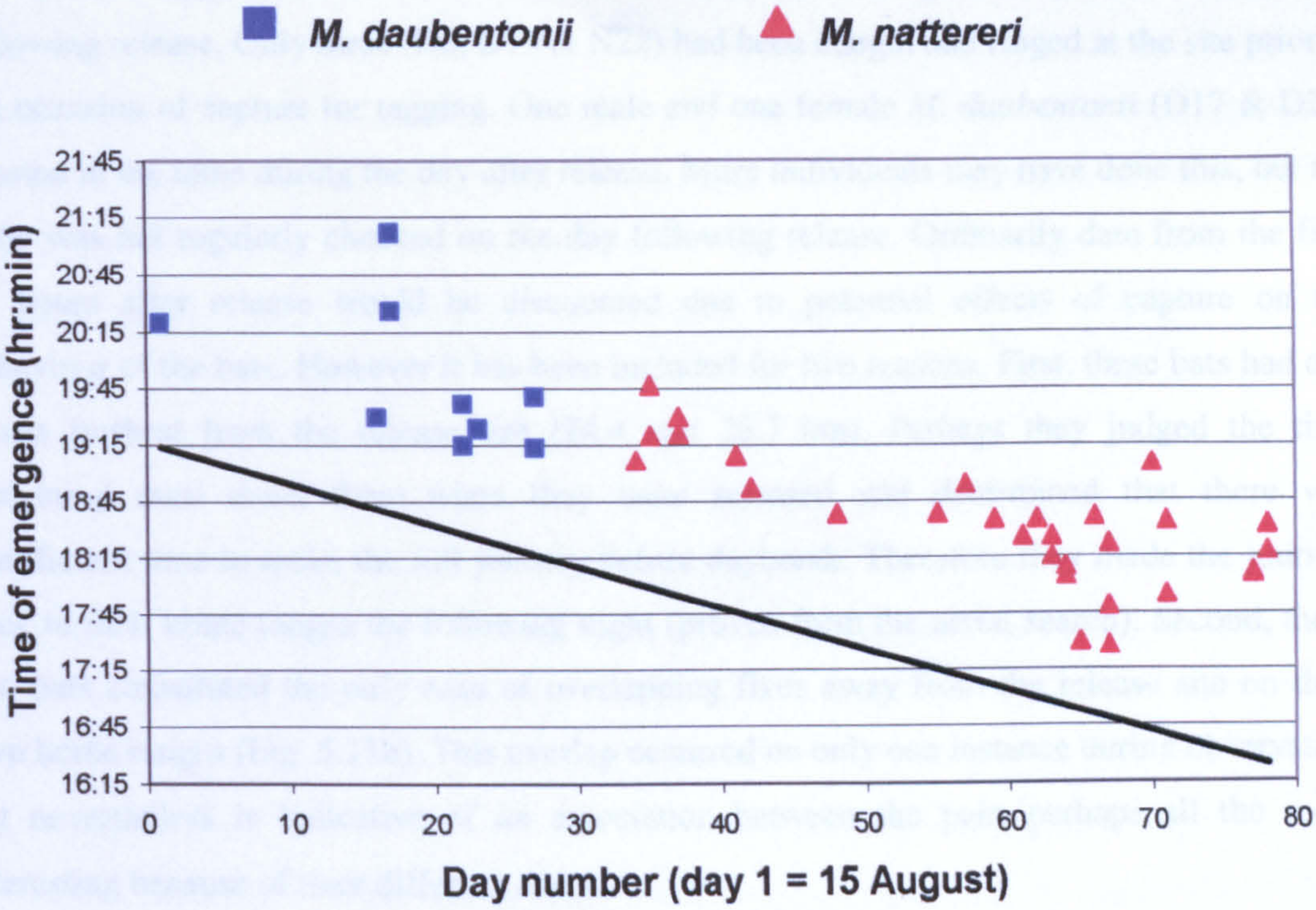
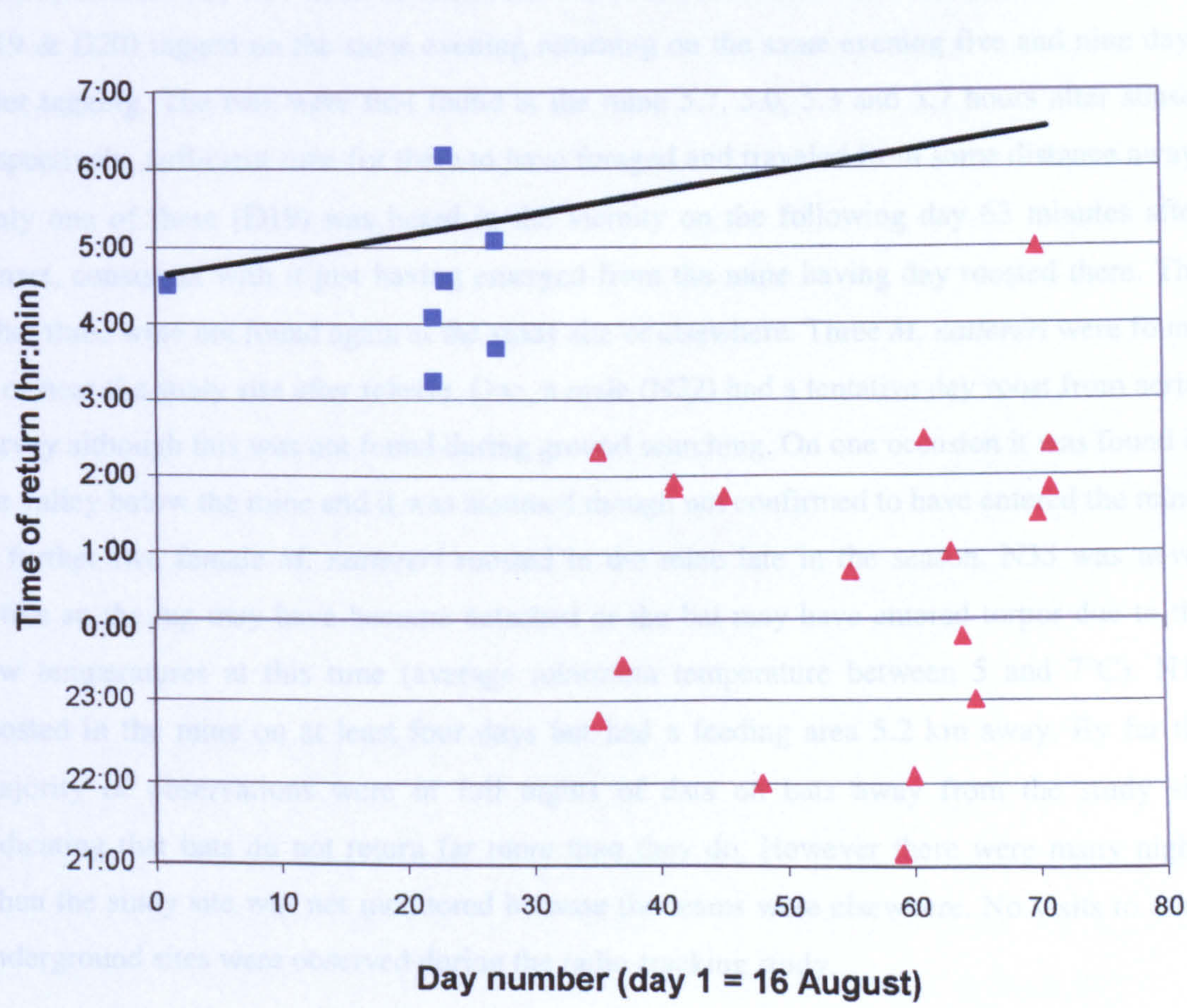


Figure 5.15. Time of return to roost for *M. daubentonii* and *M. nattereri* relative to sunrise shown by the heavy line between 16 August and 25 October.



5.3.10. Return to the release site

Overall ten tagged bats (16.9%) were found at or in close proximity to the study site following release. Only three (D8, D19 & N22) had been caught and ringed at the site prior to the occasion of capture for tagging. One male and one female *M. daubentonii* (D17 & D22) roosted in the mine during the day after release. More individuals may have done this, but the mine was not regularly checked on the day following release. Ordinarily data from the first 24 hours after release would be discounted due to potential effects of capture on the behaviour of the bats. However it has been included for two reasons. First, these bats had day roosts furthest from the release site (24.4 and 26.7 km). Perhaps they judged the time remaining until dawn from when they were released and determined that there was insufficient time to make the full journey before daybreak. Therefore they made the journey back to their home ranges the following night (proven from the aerial search). Second, these two bats constituted the only case of overlapping fixes away from the release site on their own home ranges (Fig. 5.11b). This overlap occurred on only one instance during observation but nevertheless is indicative of an association between the pair, perhaps all the more interesting because of their differing sexes.

As previously mentioned only one *M. daubentonii* made repeated visits to the study site on consecutive evenings. Four *M. daubentonii* (two male, two female) were also found in the vicinity of the study site. Each of two occasions consisted of a male/female pair (D11 & D14, D19 & D20) tagged on the same evening returning on the same evening five and nine days after tagging. The bats were first found at the mine 5.7, 5.0, 5.3 and 3.7 hours after sunset respectively, sufficient time for them to have foraged and traveled from some distance away. Only one of these (D19) was heard in the vicinity on the following day 63 minutes after sunset, consistent with it just having emerged from the mine having day roosted there. The other three were not found again at the study site or elsewhere. Three *M. nattereri* were found at or near the study site after release. One, a male (N22) had a tentative day roost from aerial survey although this was not found during ground searching. On one occasion it was found in the valley below the mine and it was assumed though not confirmed to have entered the mine. A further two female *M. nattereri* roosted in the mine late in the season. N35 was never active so the tag may have become detached or the bat may have entered torpor due to the low temperatures at this time (average minimum temperature between 5 and 7°C). N13 roosted in the mine on at least four days but had a feeding area 5.2 km away. By far the majority of observations were of full nights of data on bats away from the study site indicating that bats do not return far more than they do. However there were many nights when the study site was not monitored because the teams were elsewhere. No visits to other underground sites were observed during the radio-tracking study.

5.4. DISCUSSION

5.4.1. Success of study

I consider 61% (close to 70% for *M. nattereri*) to be a relatively high relocation rate for this type of study, where tracking was not centred at day roosts. In general radio-tracking studies following females from a nursery roost are more successful because the bats are less likely to travel great distances. However, no radio-tracking study is likely to be 100% successful because tags may fall off or malfunction, animals may move, or be difficult to follow and obtain data from. For example Henry *et al.* (2002), radio-tagged 28 female *M. lucifugus* but got complete activity data from only 16 (57%).

Following the 2000 field season I predicted that the difficulty in relocating *M. daubentonii* might have been because they traveled further on average than *M. nattereri,* so more were beyond the area searched on the ground. Following the greater experience of the 2001 field season and in particular the use of the plane, I concluded that this was the case because three individuals were relocated beyond the area searched in 2000. *M daubentonii* were more difficult to find when active due to their habit of flying low over the surface of water. Surrounding vegetation and river/canal banks masked the signal more than for a bat flying several metres above the ground. Roosts of *M. daubentonii* might also have been more difficult to locate if they were in dense woodland.

Some *M. daubentonii* were relocated or heard from the plane during the day after release and subsequently never located again. I concluded that these bats were using transient roosts, presumably as staging posts between the release site and a home range even further away than the furthest bats found here. I recommend that flights be undertaken more than 24 hours after release to avoid finding only transient roosts. A second flight some days after the first would also be beneficial but was prevented by the expense. Hicks *et al.* (2003) used an aircraft to monitor the direction bats flew on release and on the following day used two aircraft flying in opposite concentric circles toward the release site from 24 km away. They located 16 of 19 animals in an area of 12,000 km^2, all within 38 km of the release point. These improvements in aerial tracking methods would be of benefit in the study of movement of swarming bats, which I found to cover similar distances.

5.4.2. Dispersion of bats around the study site

The prediction that *M. daubentonii* travel further from swarming sites than *M. nattereri* was not statistically upheld, although 39% of tagged bats (32% *M. nattereri*, 50% *M. daubentonii*) remained unaccounted for. Many of the *M. daubentonii* probably roosted far from the study site, and were therefore difficult to relocate. There was great variation in distance traveled by

bats from the study site to day roosts, which were not evenly distributed throughout the study area. Although bats have potential to go in any direction from the swarming site their actual distribution may be constrained by factors such as habitat, roost availability or landscape features along which they can make long-distance commuting journeys (e.g. rivers). Historical records (majority pre-1990) of roosts and grounded bats of both species (obtained from Biological Record Centers and local Bat Groups) have a more complete distribution around the release site. Our skewed distributions may reflect loss of preferable foraging habitat and/or roost sites in recent years.

Flight enables the use of scattered and rare, but preferred roosts, even when at some distance from feeding sites (Bradbury, 1977b). The same can be said of swarming sites, whereby individuals normally dispersed in an environment can, by virtue of their mobility, visit distant sites within a short time. Flight speed of *M. nattereri* has previously been estimated as 15.5 km/hr (Seimers *et al*, 1999) and in this study at 14.5 km/hr, thus even the most distant home range in this study would have been reached from the study site in around an hour and a half.

The maximum range of 26.7 km was revealed to be an underestimate of the actual range over which bats are drawn to the study site. Recapture of ringed bats has shown that they visit the study site during swarming from more than 35 km away. The potential catchment area is therefore in the region of 4118 km^2 and may be even greater still. Richardson (1989) recorded movement of a *M. daubentonii* 19 km away from a tunnel site (probably used for swarming), within the range demonstrated here. The longest distance recorded in Europe is 260 km (Urbańczyk, 1991 cited by Bogdanowicz, 1994). The previous longest recorded movement of *M. nattereri* in Britain was 24 km (Stebbings, 1991) and in Europe 62 km (Bels, 1952 cited in Corbet & Harris, 1991), but a recent find of a female *M. nattereri* ringed during swarming in north Yorkshire, at a nursery roost 63 km away has taken the record (Lane, 2003). Bats of similar size have made greater journeys, for example, *M. sodalis* travels 467 km between winter and summer roosts (Kurta & Murray, 2000).

The term 'catchment area' implies that bats are present on their home ranges before swarming also. Unfortunately, with the exception of one male *M. daubentonii,* no bats were tracked prior to and after swarming. However in summer 2002 a female *M. nattereri* ringed during swarming at Box was located at a summer roost used by one of the tagged females the previous year. This was confirmed to house a sizeable nursery colony of about 80 individuals. Bats of both sexes may travel from familiar home ranges, where they spend the summer, to the study site and back again. Females from two other colonies, one to the east and another to

the west of the study site, have also visited the site during swarming. This confirms that bats from different natal groups are gathering from a wide area at a central point.

5.4.3. Habitat preferences

M. daubentonii roosts were exclusively in trees, predominantly oaks, confirming the preference found by Boonman (2000). The bats did not always use the oldest trees available in the parkland, highlighting the importance of preserving younger roost trees alongside protecting ancient trees. Smith and Racey (2002) found that one third of day roosts of summer colonies of *M. nattereri* were in buildings and two-thirds in trees, however I found a more even split (11:13) between buildings and trees and also use of the mine as a day roost late in the season. After breeding, females in particular are probably less constrained to certain roost types because they no longer require nurseries for their young.

It is likely that *M. daubentonii* used major river corridors for commuting, as does *Miniopterus schreibersii* (Serra-Cobo *et al.*, 2000) because all roosts were situated within several hundred meters of major waterways (including Rivers Avon and Frome and the Kennet & Avon Canal) (also found by Speakman *et al.*, 1991 for *M. daubentonii* summer roosts in Scotland) and this species is particularly associated with riparian habitats (Rydell *et al.*, 1994). The mix of habitats found on large country estates (parkland, woodland and open water) appears particularly favourable to *M. daubentonii.* Glendell & Vaughan (2002) also found that *Myotis* bats selected water and plantation woodland habitats within landscape parks, and most activity over water was attributed to *M. daubentonii.*

M. nattereri demonstrated considerable diversity in roost type and in habitat found around roosts, although arable and pastural habitats were found more around roosts than expected from random suggesting that *M. nattereri* preferentially roost in rural rather than urban areas. The dominant habitats for foraging (woodland, arable and pasture) comprised on average 85% of the area within 2 km of roosts (min 65.5%, max 95%). Mobility gives bats access to mosaics of habitat (Fenton, 1997) and for *M. nattereri* mixed agriculture, rather than exclusively arable or pastural, may be preferred. This likely reflects the general land use in this area of Wiltshire, making this region of the country particularly suitable for this species. To the north there is a dominance of arable and to the west is pastural agriculture and a major urban area.

A shift in relative importance of habitats occurred between the large and small-scale analyses. In particular the woodland habitat gained much greater importance as a component of home ranges. Although woodland was not found in great amounts within 2 km of roosts, it was

used most in proportion to its availability on home ranges, indicating the importance of this habitat type for the species. *M. nattereri* mainly used woodland, pasture, arable and open water habitats for foraging, consistent with the findings of Smith & Racey (2002) and the general findings for *Myotis* group bats by Glendell & Vaughan (2002). Amenity land, associated with large urban areas such as towns and cities, was used least. Much of the urban category in the analysis comprised rural dwellings, such as farmsteads and small villages, which are important for some *M. nattereri* as roost sites. Because none of the habitats was significantly preferred over an adjacent habitat in the analyses, these findings suggest that *M. nattereri* roost in an area with a matrix of rural habitats, and once there they utilize most of the available habitats and are not particularly selective, although most rely on woodland for feeding in addition to arable and pastural field edges. Further analyses could separate out the different categories of built-up land.

5.4.4. Home ranges and nightly activity budgets

M. daubentonii were strongly faithful to the same foraging site each night. Each bat had only one large body of water (5.5. – 14.5 ha) available near its roost and this was exploited for foraging. Foraging site fidelity has previously been documented in this species (Richardson, 1985; Swift & Racey; 1983). The distances traveled from roosts to foraging areas were comparably small in my study.

During their nightly movements female *M. nattereri* ranged on average at least 1 km further than males both to the edge of their range and to their foraging areas. This suggests that females may in some way be constrained to certain roosts away from favoured feeding areas (perhaps in or near traditional parturition roosts) whereas males roost much closer to their feeding areas. *M. nattereri* also displayed considerable fidelity to feeding areas as found by Seimers *et al.* (1999). Range sizes were similar to those recorded by Seimers *et al.* (1999) and Smith and Racey (2002).

Emergence times agreed with those previously given for *M. daubentonii* (84 minutes post-sunset) and *M. nattereri* (75 minutes post-sunset) by Jones & Rydell (1994). Swift (1997) gave an emergence time of 56 minutes post-sunset for *M. nattereri* in Scotland. Perhaps there was an effect of latitude on emergence time, although this would be contrary to observations by Jones & Rydell (1994) that bats at lower latitudes emerge earlier because the length of the twilight period is shorter in duration. The earlier mean time of emergence for female *M. nattereri* (and perhaps the reason why overall mean emergence of *M. nattereri* was earlier than *M. daubentonii*) can largely be attributed to bat N13 which emerged early (mean 39 minutes post-sunset) from its roost in the mine, perhaps because of the distance required to

travel to the foraging site, whereas other bats roosted much closer to where they fed. While females might be expected to emerge earlier than males while under energetic stress of lactating (Duvergé *et al.*, 2000), this difference would not be expected between August and October.

5.4.5. Visitation of swarming sites

Lack of movement to other underground sites and return to the study site by some individuals during observation suggests that bats are faithful to one swarming site. Similarly, Humphrey and Cope (1976) found little evidence for visitation of other caves in Indiana and Kentucky by *M. lucifugus*. There was great variation in the degree of return observed. One male *M. daubentonii* visited on consecutive evenings, and contrary to expectation it spent much time in the tree canopy and only went underground sporadically for short durations. Males and females re-visited the site with equal frequency and the discovery of male and female pairs arriving together at the study site or traveling together from the study site to local overlapping home ranges, lends great support to the hypothesis that swarming sites are mating sites or concerned with the location of mates. Other individuals, however, even those at closer proximity to the study site, were never observed to return despite many nights of observation. Furmankiewicz (2002) observed male *P. auritus* making round trips of up to 14 km in one evening to spend a few hours at a swarming site.

5.4.6. Conservation implications

Protection and enhancement of habitats preferred for roosting and foraging by swarming species will help secure their survival. *M. daubentonii* primarily requires parkland with open water and *M. nattereri* requires woodland in a matrix of rural habitats. The preservation of the swarming site studied here and of others in the region will benefit bats over a large area of southern England. If, as suspected, swarming sites are foci for mating activity in these and other *Myotis* species, the large catchment areas will surely favour out-breeding, a topic investigated further in Chapter 7. Maintenance of genetic variability in populations by outbreeding has been linked to increased survival at an individual level (Rossiter *et al.*, 2001). At a population level, increased heterozygosity might increase the chance of the species surviving a large reduction in population size. The possibility that bats from widely dispersed colony groups are faithful to one swarming site raises important issues about site protection. Bats from a large area might be less likely to find mates or hibernation sites should swarming sites be destroyed. Any estimate presented here is likely to be an underestimate of seasonal, annual and life-time range requirements (O'Donnell, 2001) and so conservation areas should be large to encompass more than just roosts.

CHAPTER 6

REPRODUCTIVE STATUS AND BODY CONDITION OF *MYOTIS* AND *PLECOTUS* BATS DURING SWARMING

6. REPRODUCTIVE STATUS AND BODY CONDITION OF *MYOTIS* AND *PLECOTUS* BATS DURING SWARMING[1]

SUMMARY

Temperate zone vespertilionid bats are seasonal breeders. Females begin oestrus following weaning of the young in late summer. Males undergo spermatogenesis during summer and spermatozoa are stored in the cauda epididymides prior to mating, which in most species occurs from autumn through to the following spring. Females also store spermatozoa over winter in the reproductive tract. Ovulation and fertilization occurs in the spring after emergence from hibernation.

Observations of changing reproductive condition in male *Myotis* and *Plecotus* bats were made during catching surveys at a swarming site. Progression from sexual inactivity to spermatogenesis to sperm storage in males was indicated by changes in the external appearance of the testes and epididymides. Parous and non-parous females were distinguished by the condition of the nipples. Change in body mass during the year was monitored via indices of body condition calculated from measurements of body mass and forearm length.

The timing of sexual readiness in males of different *Myotis* species was synchronous with the time of their peak swarming activity, lending support to the theory that swarming concerns the location of mates and mating. The epididymides remained distended in some individuals until April suggesting that sperm were potentially available for matings throughout hibernation and in the spring.

30-40% of juveniles of each species may become sexually mature in their first autumn. The density of pigment in the tunica vaginalis was not a reliable indicator of previous sexual maturity in the species studied. Annual development of the sexual organs in preparation for mating occurs earlier in those males with good body condition than in those with poor body condition.

Parous adult females had higher body condition during the swarming season than non-parous females. Females of all species, except *M. brandtii,* had longer forearms than males.

[1] A paper based on this chapter is in preparation for publication under the title "Reproductive and body condition of male *Myotis* and *Plecotus* bats during swarming". G. Jones is co-author.

6.1. INTRODUCTION

The explanation for swarming that has received most support in recent years is that it is connected with finding mates and mating (Fenton, 1969, Thomas *et al,* 1979). Temperate zone bats become ready for mating during autumn and social behaviour comprising calling, chasing and copulating has been observed during swarming at underground sites (Racey *et al.*, 1987; Thomas *et al.,* 1979). Capture of bats at swarming sites allowed me to study changes in the external reproductive morphology of males over time and to compare patterns of sexual readiness and time of onset of sexual maturity between males of the different species. Comparisons were made between body masses and forearm lengths of male and female adults and juveniles during swarming also.

6.1.1. Male reproductive anatomy

The structure of the male reproductive organs in the Chiroptera follows the normal mammalian pattern of paired testes and accessory glands (Krutzsch, 2000). Specializations by family and also by geographical region exist, however, both in the structure of the reproductive organs and in the reproductive cycle. Temperate zone microchiropteran males have only one period of sexual readiness per year and development of the primary sexual organs (testes) is asynchronous with that of the secondary organs (accessory glands) (Gustafson, 1979; Krutzsch, 2000). Spermatogenesis occurs in the testes during the summer and reaches a peak in late August (Wimsatt, 1969). Spermatozoa are subsequently transferred for storage in the cauda epididymides before mating begins in autumn (Fig. 6.1). The testes then regress fully while the epididymides remain distended until the sperm are removed through mating. The weight of the testis correlates with spermatogenesis and the weight of the epididymis correlates with sperm storage (Neuweiler, 2000). Sperm can be stored in the epididymides and remain viable throughout the winter (Racey, 1973). This temporal separation of reproductive activity is presumably a specialization to a mating season interrupted by hibernation (Wimsatt, 1969). Males are considered to be ready to mate when the testes have regressed and the epididymides are distended (Neuweiler, 2000). Outside the mating season both the testes and epididymides return to pre-pubertal size.

The morphology of the testes and epididymides of vespertilionid bats can be observed easily with the bat in the hand and no invasive exploration is necessary. Therefore assessing the sexual maturity of a male bat can be performed rapidly alongside taking other records, such as body mass and forearm length.

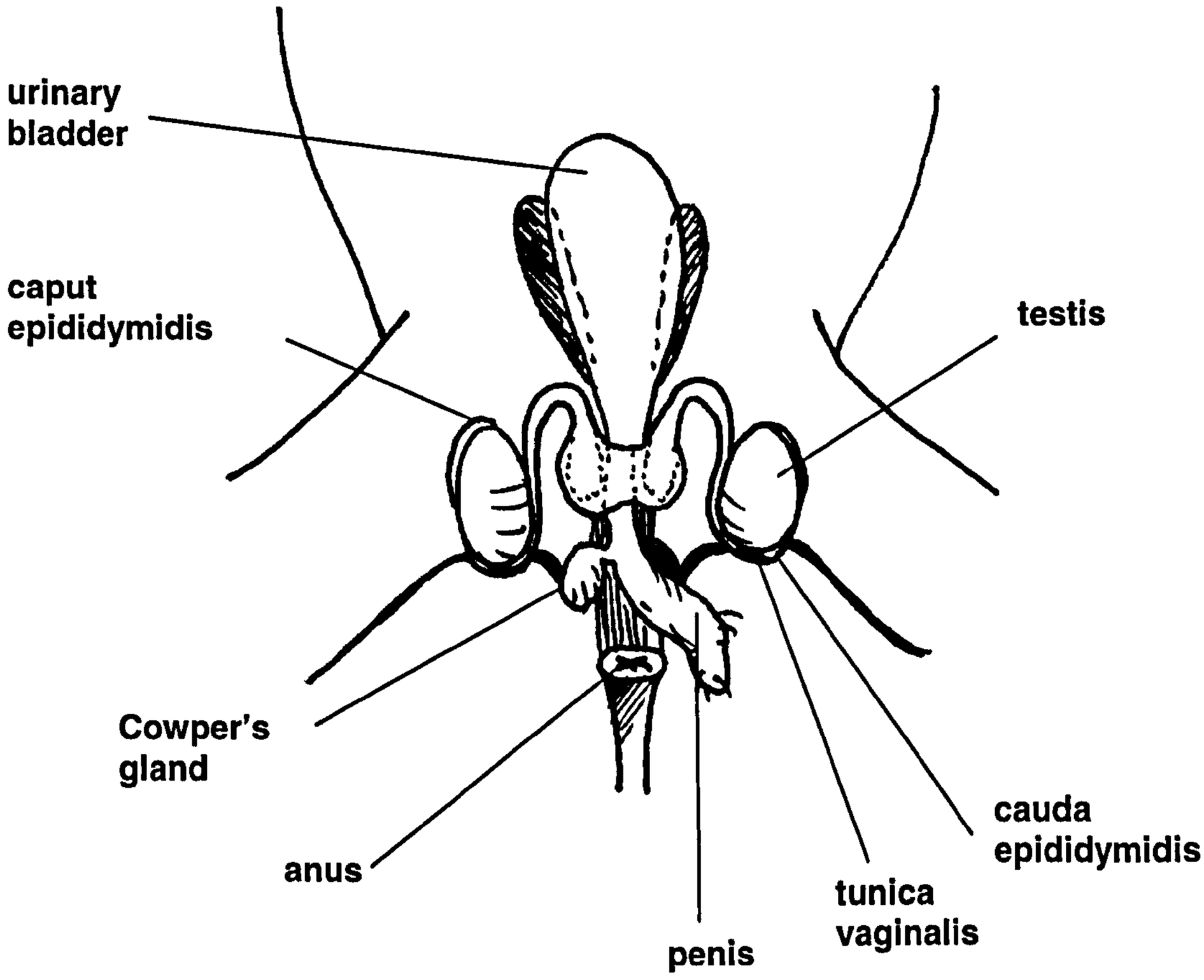


Figure 6.1. Structure of the male reproductive tract in the family Vespertilionidae (based on *Lasiurus borealis* after Krutzsch, 2000).

The colour of the tunica vaginalis (Fig. 6.1), the melanin-pigmented sheath surrounding the epididymis, has been used to signal prior sexual maturity in some species (e.g. *Pipistrellus pipistrellus* by Racey, 1974a), but has been found less useful for others (e.g. *Plecotus auritus* by Entwistle *et al.*, 1998; and *Myotis daubentonii* by Kokurewicz & Bartmańska, 1992). The role of this sheath is unknown but believed to be connected with thermoregulation and sperm viability (Krutzsch, 2000).

Compared with females, male bats have been the subjects of relatively few investigations (Krutzsch, 2000) and despite the large size and widespread distribution of the family Vespertilionidae, the male reproductive cycle has been described in detail for relatively few British species (e.g *Nyctalus noctula* by Racey, 1974b; *Pipistrellus pipistrellus (sensu lato)* by Racey & Tam, 1974; *Plecotus auritus* by Entwistle *et al.*, 1998; Speakman & Racey, 1986 and Stebbings, 1966). In the genus *Myotis* most is known about the reproductive cycle of the north American species *Myotis lucifugus* (Gustafson, 1979; Racey *et al*, 1987).

This study permits comparison of changes in reproductive condition among males of all five British species of *Myotis* bats and *P. auritus*. I hypothesise that the timing of sexual activity will be closely connected with timing of swarming in the different species.

6.1.2. Annual mass change in hibernating bats

Hibernating mammals typically undergo massive annual variation in body mass. They must lay down fat reserves in autumn to meet their energy demands during the period of hibernation. During hibernation the fat stores are depleted and they emerge in spring at a lighter body mass than when they entered hibernation. Annual gains and losses in *Myotis* bats have been calculated as between 15 and 40% of spring and autumn body mass (Harrje, 1994; Johnson *et al.*, 1998; Kunz *et al.*, 1998) and 22% in *Plecotus auritus* (Stebbings, 1970).

Body condition is the ratio of body mass to forearm length, which indicates whether a bat is heavy (in 'good' body condition) or light (in 'poor' body condition) for its size. Body condition of male bats has previously been connected with their degree of sexual development (Entwistle *et al.*, 1998; Speakman & Racey, 1986), where bats with good body condition have more advanced sexual development than those in poorer body condition. It has also been suggested that the fattest males at the onset of hibernation may have a reproductive advantage because they will be able to arouse more frequently to engage in winter copulations (Kunz *et al.*, 1998). However, males may actually lose mass during the pre-hibernation period, possibly due to the energetic demands of mating (Lundberg & Gerell, 1986) and have a very short time in which to gain mass before hibernation. Females in good body condition may have an advantage if they retain sufficient reserves on emergence from hibernation to have rapid ovulation and successful gestation (Kunz *et al.*, 1998). Adults of both sexes will probably weigh more than juveniles (Kunz *et al.*, 1998).

6.1.3. Sexual dimorphism in size of vespertilionid bats

Female vespertilionid bats tend to be larger than males (Myers, 1978; Williams & Findley, 1979). One possible explanation for this dimorphism in size is that larger females have a selective advantage because they are better able to fly whilst carrying their young during pregnancy and after birth (Myers, 1978). Alternatively larger females might be better able to control their body temperature and length of gestation, may be able to store more fat and may have a greater range of prey available to them (Williams & Findley, 1979). The studies by Myers (1978) and Williams and Findley (1979) were literature reviews of predominantly New World (North and South American) species. It is likely that the same selective forces will have operated on vespertilionid bats in Britain and that females will on average be larger

than males. Such dimorphism has previously been documented for *M. daubentonii* (Speakman, 1991; Stebbings, 1977), *M. nattereri* (Stebbings, 1977), *P. auritus* (Stebbings, 1967; 1970) and *M. mystacinus* (Stebbings, 1977).

The specific aims of this chapter are:

1. To describe the changes in the male reproductive organs of *Myotis bechsteinii, M. brandtii, M. daubentonii, M. mystacinus, M. nattereri* and *Plecotus auritus* during the swarming season.
2. To determine whether spermatozoa might remain available for spring matings.
3. To investigate when juveniles become sexually mature.
4. To assess whether the degree of pigmentation of the tunica vaginalis is a reliable indicator of prior sexual maturity.
5. To study change in body condition of males during swarming (pre-hibernal period) and to see if body condition influences the degree of development of the reproductive organs.
6. To study body condition of females during swarming to see if adult parous individuals are heavier than juvenile or non-parous individuals.
7. To compare body size of males and females.

6.2. METHODS

6.2.1. Assignment of reproductive condition

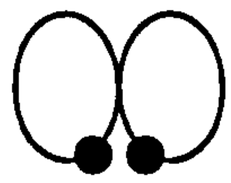
Male bats were caught during survey work at Box stone-mine between 1995 and 2002 by G. Jones (1995 to 1998) and myself (1999 to 2002) (see Chapter 2 for details of capture methods). The size of the testes and the size and pigmentation of the cauda epididymides were recorded (Racey, 1982; Racey, 1988; Thomas *et al.*, 1979) for each individual. The testes and epididymides were each graded as not obvious, small, medium or large. The epididymides were described as black, white, speckled or white with a black tip. Drawings and photographs recorded the different configurations of testis and epididymidis size and pigmentation associated with different stages of sexual maturity. Four stages of reproductive condition were ascribed to individuals based on the descriptions of testis and epididymidis size (Plate 6.1). In summary, these are:

Condition 0: Sexually inactive or immature

Showing no signs of testis growth or epididymal distension
Individual either 'inactive' (sexually mature but testes quiescent) or 'immature' (not yet sexually mature)

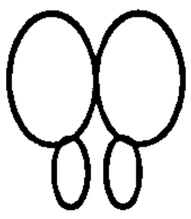
Condition 1: Spermatogenesis

Testis enlarged, no epididymal distension obvious



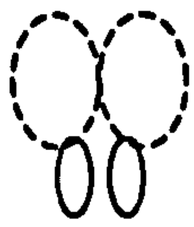
Condition 2: Sperm transfer

Testis enlarged, epididymis distended.
Assumed transfer of sperm from testes to epididymides.



Condition 3: Sperm storage

Testis not obvious (regressed), epididymis distended



The conditions of the nipples was noted for females as an indication of whether they had given birth and lactated that summer (parous) or were immature and had not previously given birth (nulliparous) (Racey, 1974a; Racey, 1988).

6.2.2. Assignment of age

Bats of both sexes were assigned juvenile (<1 year old) or adult (>1 year old) status on examination of the epiphyseal joints in the finger bones (Anthony, 1988; Racey, 1974a). Separation of juvenile from adult individuals was problematic during the autumn and became increasingly difficult as the season progressed because the epiphyses become increasingly

fused with the diaphyses (Thomas *et al.*, 1979). Some bats were fitted with rings as mentioned in Chapter 4.

6.2.3. Calculation of body condition indices

Each bat was weighed with a spring balance to 0.1 g, and the length of the right forearm was measured with plastic calipers (to 0.1 mm). A relative index of body condition (BCI) was calculated for each individual according to the method used by Entwistle *et al.* (1998). The relationship between forearm length and body mass was described for all individuals of each sex of each species separately by the equation of a fitted regression line (Table 6.1). This equation was used to calculate expected body masses for each bat based on its forearm length. The residual (observed mass – expected mass) was taken as an index of relative body condition. A positive value indicated a body mass above that expected for that forearm length and a negative value indicated a body mass below that expected. In cases where regressions were not significant (probably due to small sample sizes) a simpler measure of absolute body condition (individual body mass/forearm length) was used.

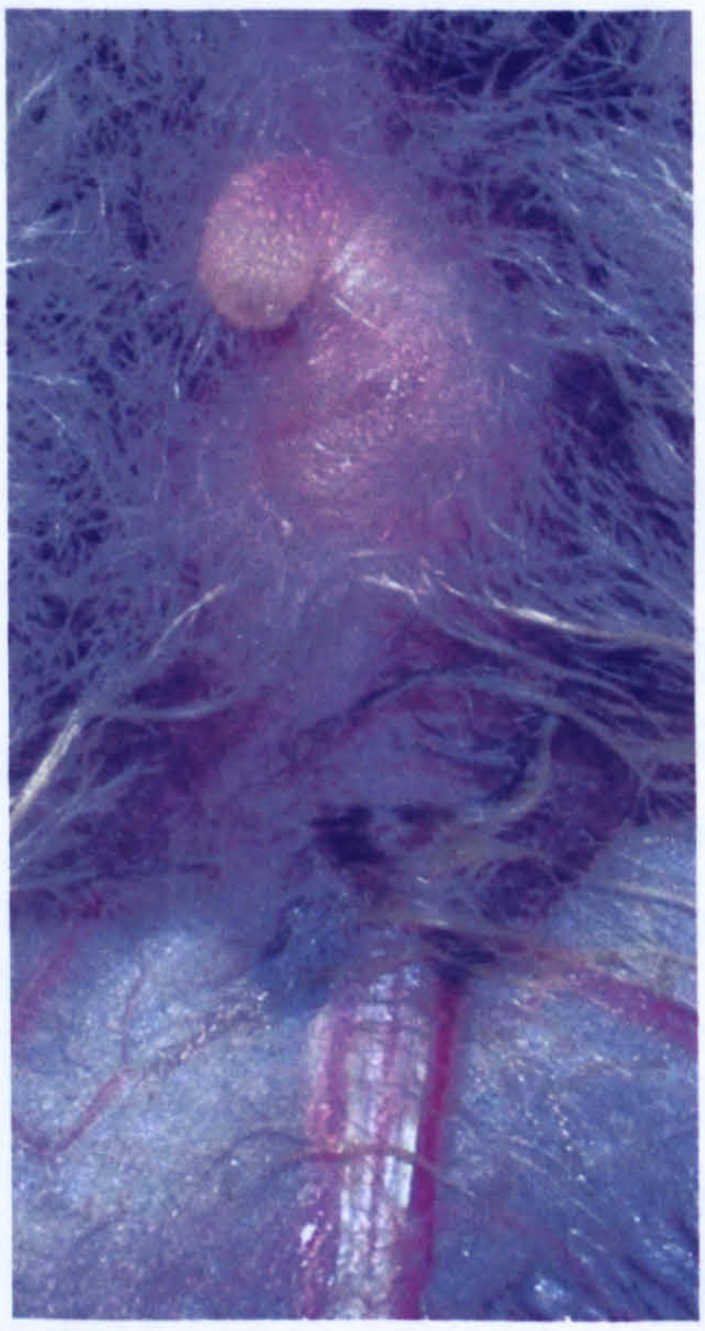
Table 6.1. Results of significant regressions describing the relationship between forearm length (fore) and body mass for both sexes of each species[1]. The equations were used to calculate expected masses for calculation of relative body condition indices for analysis.

Species and sex	Regression equation Mass (g) Forearm (mm)	*F*	r^2	n	*P*
Md ♂	Mass = (0.217*fore) + 0.15	22.22	0.046	462	<0.0001
Mm ♂	Mass = (0.176*fore) - 0.81	8.41	0.047	192	0.004
Mn ♂	Mass = (0.199*fore) - 0.10	52.26	0.059	833	<0.0001
Md ♀	Mass = (0.413*fore) - 6.47	13.89	0.089	145	<0.0001
Mn ♀	Mass = (0.207*fore) – 0.20	20.12	0.058	330	<0.0001

[1]Md = *M. daubentonii*, Mm = *M. mystacinus*, Mn = *M. nattereri*

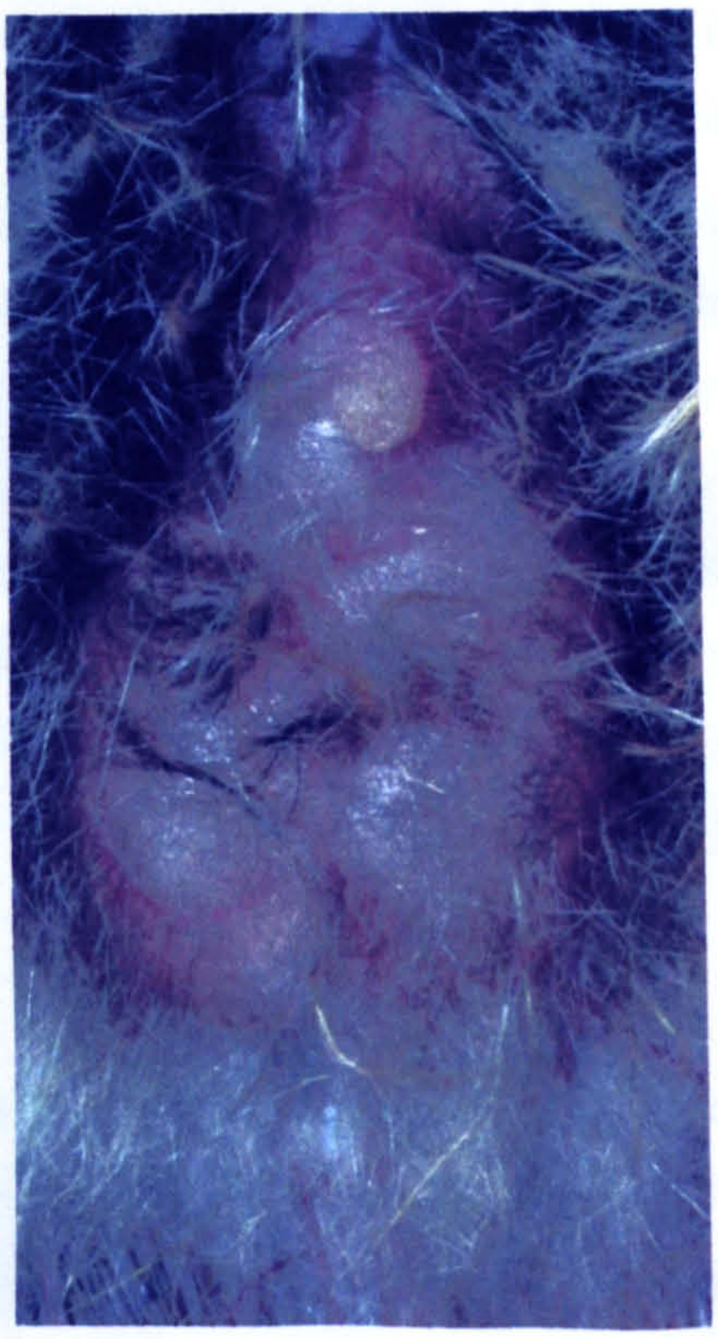
BCI was mostly not normally distributed hence non-parametric statistics (Mann-Whitney U and Kruskal-Wallis tests) were used in comparing body condition between months and between reproductive conditions. Months or body categories with a sample size of less than five were excluded from the analyses.

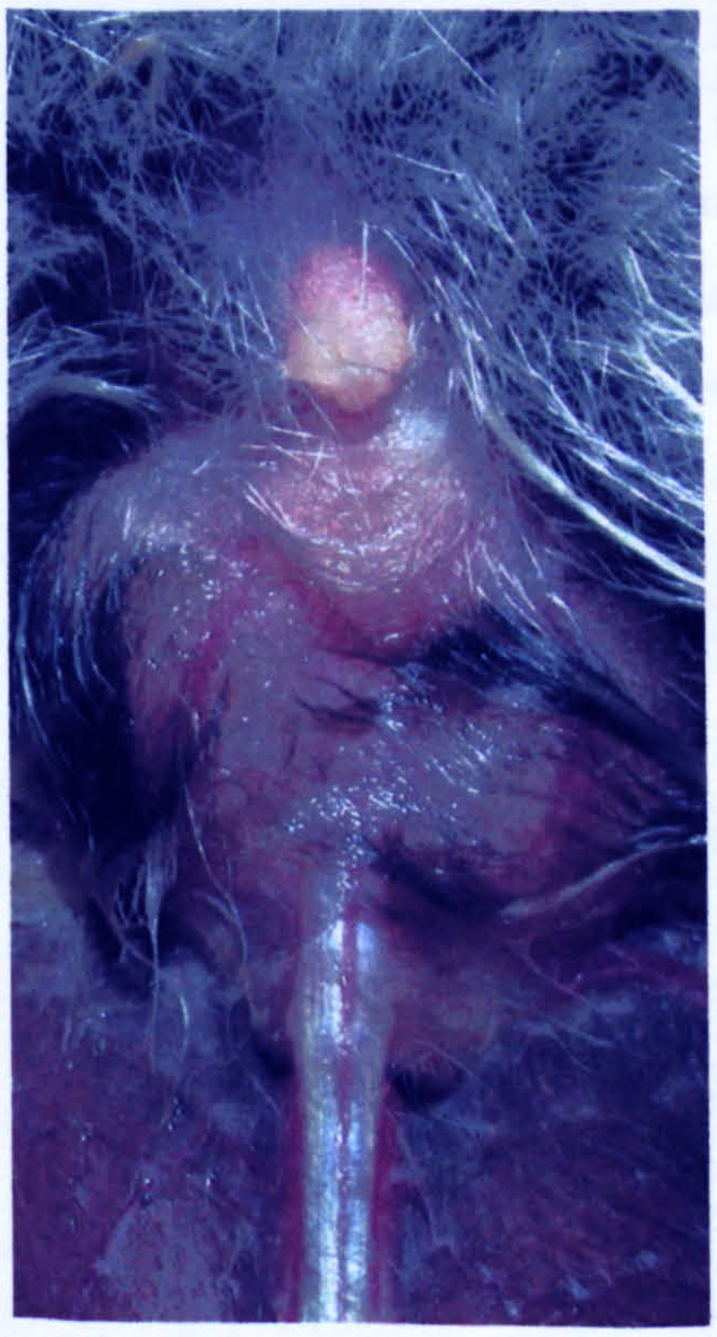
Plate 6.1. Photographs showing the different stages of testicular growth and epididymal distension during the reproductive cycle of male *Myotis* bats.



Left:
Condition 0
No obvious testes
Small black epididymides
(*M. daubentonii*)

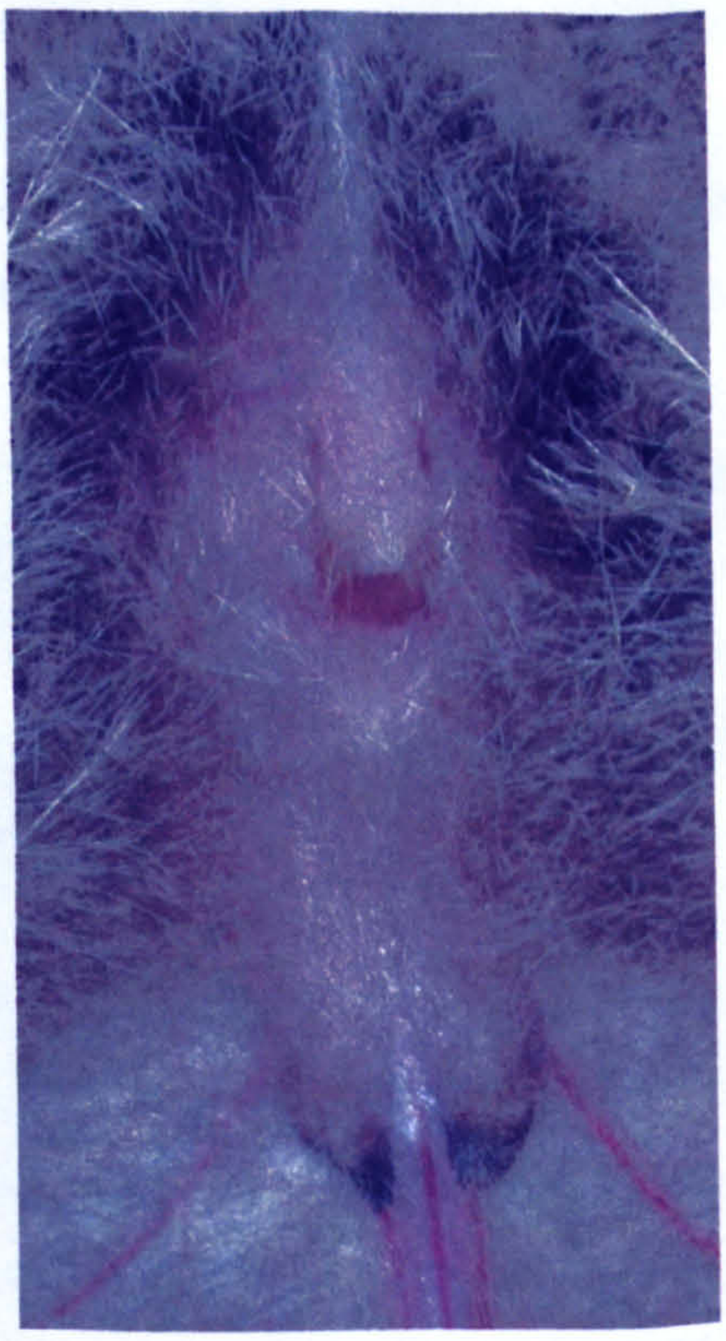
Right:
Condition 1
Large testes
No obvious epididymides
(*M. nattereri*)





Left:
Condition 2
Medium to large testes
Distended epididymides with black tip
(*M. nattereri*)

Right:
Condition 3
No obvious testes
Distended epididymides with black tip
(*M. daubentonii*)



6.3. RESULTS

6.3.1. Reproductive timing in males

A total of 947 bats of six species were scored for testis and epididymis size during this study, comprising 37 *Myotis. bechsteinii,* 100 *M. bechsteinii,* 332 *M. daubentonii*, 138 *M. mystacinus,* 312 *M. nattereri* and 28 *Plecotus auritus*. The majority of captures (92.2%) were made during the autumn swarming season and a few (7.8%) were during spring. Time of maximal testis growth with no visible epididymal distension (Condition 1) differed among the species studied (Fig 6.2). A large proportion of *M. brandtii* and *M. daubentonii* caught in July were obviously undergoing spermatogenesis at that time. Other species were not captured in July thus it cannot be confirmed whether or not they began spermatogenesis then also. However, it is likely for *M. bechsteinii*, *M. mystacinus* and *P. auritus* because one third of males caught in August already exhibited Condition 2 (presumed sperm transfer). Only *M. nattereri* continued to exhibit Condition 1 beyond the end of August. Spermatogenesis continued in this species until at least late September. Spermatogenesis had ceased in all other species before the start of September.

Reproductive condition of males was examined at greater resolution (half-monthly intervals) for the four species with greatest sample sizes: *M. brandtii, M. mystacinus, M. daubentonii* and *M. nattereri*. Mean reproductive condition was calculated for all males scored for reproductive condition within each time category (Fig. 6.3). A steady increase in mean reproductive condition was seen in all species during the first half of the swarming season coinciding with spermatogenesis, progression to sperm transfer and eventually sperm storage. A clear order can be seen in timing of these processes with *M. brandtii* attaining sexual condition first, followed by *M. daubentonii*, then *M. mystacinus* and lastly *M. nattereri* (Fig. 6.3). For *M. brandtii* the data for early September may be anomalous due to the small sample size or it could be a real decrease consistent with arrival of juveniles at the site. In the remaining species a slight decrease in mean reproductive condition is seen around late September and early October. This apparent decrease is most likely explained by the arrival of many juveniles at that time which increased the proportion of bats showing Condition 0 and consequently decreased the mean reproductive condition.

By ringing bats I could follow the sexual progress in individuals. Transfer of sperm to the epididymides took place at different times in different years in some individuals. For example one *M. daubentonii* caught on 16 August 2000 was scored Condition 1 (with large testes and no obvious epididymides) yet on 12 August 2001 it was scored Condition 2 (with small testes and medium white epididymides), so sperm transfer began earlier in 2001 than in 2000. This trend was not consistent for all individuals at a particular time indicating that whatever

influences the timing of spermatogenesis and transfer of sperm to the epididymis does not affect all individuals equally and at the same time.

Although sample sizes are small for all species in the spring months (0 for *M. bechsteinii*), it was clear that the epididymides of many males remained distended throughout hibernation and therefore sperm were still potentially available for spring matings (Fig. 6.2). During spring most records were of small or medium-sized epididymides indicating that some sperm had been removed by mating (or resorbed). No testes were found enlarged during March and April.

6.3.2. Onset of sexual maturity in males

Some non-reproductive individuals of all species were captured throughout the swarming season (Condition 0). 63.2 to 83.4% of bats identified as juveniles (in their first year) were in Condition 0 (Table 6.2). For all species, however, a small proportion of juveniles exhibited at least one of the sexually mature conditions (Table 6.2). Due to difficulty in assigning age to individuals during the autumn these age ratios must be viewed with caution. It is all too tempting to assign any bat found during autumn with testicular or epididymal distension as an adult, however these results suggest that some juveniles, at least may become sexually mature in their first year within three to four months of birth.

Table 6.2. Proportion of juveniles (%) of each species[1] in each reproductive condition. n = sample size. For example 83.4% of juvenile *M. bechsteinii* were in Condition 0.

Condition	Species					
	Mbe	Mbr	Md	Mm	Mn	Pa
0	83.4	75.0	63.2	77.2	82.0	75.0
1	0.0	0.0	5.3	1.7	5.6	0.0
2	0.0	0.0	21.0	5.3	7.9	0.0
3	16.6	25.0	10.5	15.8	4.5	25.0
n	6	16	76	57	89	4

[1] Mbe = *M. bechsteinii*, Mbr = *M. brandtii*, Md = *M. daubentonii*, Mm = *M. mystacinus*, Mn = *M. nattereri*, Pa = *P. auritus*

6.3.3. Pigmentation of the tunica vaginalis

Some individuals retained the black pigmentation of the tunica vaginalis beyond their first season of sexual activity. Three *P. auritus* were recorded with speckled or black epididymides after recapture, having been sexually mature in a previous year. The

epididymides of *M. daubentonii* were mostly white, but some had a black tip (16.1% of recaptures, n = 31) (see Plate 6.1. Condition 3). Similarly, most recaptured *M. nattereri* were recorded with white epididymides, particularly in Conditions 2 and 3 (71.4% of recaptures, n = 35). Bats that had been mature in previous years were sometimes recorded later with small black epididymides before the epididymides had distended again (Condition 1). No *M. bechsteinii* had black or speckled epididymides when re-captured; however the sample size was only three. *M. mystacinus* and *M. brandtii* were not ringed so there is no individual data, however it is of note that *M. mystacinus* in particular often had speckled epididymides, even when they were distended. Asymmetry was occasionally seen in both the size and degree of pigmentation of the epididymides of some individuals, for example, the two sides might be of different length and one might be wholly white and the other white with a black tip. Usually the asymmetry was not great enough to create difficulty in assigning the individual to a reproductive condition category.

6.3.4. Change in male body condition index with time

For the three species for which it could be calculated (*M. daubentonii*, *M. mystacinus* and *M. nattereri*) mean relative body condition of male bats was low on emergence from hibernation in March and April and generally increased from spring to autumn (Fig. 6.4). Relative body condition of male *M. daubentonii* differed among months (Kruskal-Wallis $H = 44.88$, d.f. = 5, $P < 0.0001$) and was particularly low in July. It remained stable during August and September and then increased steeply to November. *M. nattereri* were in poorer body condition in August than they had been in April but rapidly improved condition between August and September. Body condition remained constant into October and then a further increase was seen in November. Body condition of *M. nattereri* differed significantly among months (Kruskal-Wallis $H = 39.19$, d.f = 5, $P < 0.0001$). *M. mystacinus* improved body condition gradually between August and October but the differences were not significant (Kruskal-Wallis $H = 0.69$, d.f. = 3, $P = 0.875$). Two individuals captured in November were considerably heavier than those caught in October.

Absolute body condition for an individual at any point in time (body mass/forearm length) was calculated for *M. bechsteinii*, *M. brandtii* and *P. auritus* (Fig. 6.5). Mean body condition of male *M. bechsteinii* did not differ significantly between August and September (Kruskal-Wallis $H = 0.30$, d.f. = 1, $P = 0.583$). Body condition of male *M. brandtii* did differ significantly among months (Kruskal-Wallis $H = 30.46$, d.f. = 3, $P < 0.0001$), with a shallow increase from April to July and a steep increase from July through August to September (Fig. 6.5). Body condition in *P. auritus* was lowest in September and highest in October however

the difference between months was not statistically significant (Kruskal-Wallis $H = 1.78$, d.f. $= 2$, $P = 0.411$).

6.3.5. Reproductive and body condition in males

Kruskal-Wallis tests were performed for each species during each month to test whether males showing more advanced reproductive conditions were in better body condition than those in less developed reproductive conditions. Monthly analyses were carried out in an effort to control for the seasonal changes in body condition consistent with preparation for hibernation. For example an immature male showing no reproductive development might be in better body condition in October than a reproductively active male in August because it had laid down fat reserves for hibernation and this would influence the results if all data were considered together. Sample sizes were judged to be insufficient for *M. bechsteinii* and *P. auritus*. Relative body condition was used for *M. daubentonii, M. mystacinus* and *M. nattereri;* actual body condition was used for *M. brandtii* for which only August had a large enough sample size. A statistical difference in body condition at different reproductive conditions was found in five cases (Table 6.3).

Table 6.3. Results of Kruskal-Wallis tests comparing body condition of male bats of four species[1] in each reproductive condition during different months of the swarming season. Reproductive condition has been ranked from highest to lowest median body condition. Tests were only performed where sample sizes were greater than 5 in all conditions[2].

Species	Month	*H*	d.f.	*P*	Ranked Body Condition
Mbr	August	8.24	3	0.041	C3 > C1 > C2 > C0
Md	August	11.20	3	0.011	C1 > C3 > C2 > C0
	September	2.84	2	0.241	C3 > C2 > C0
	October	1.33	1	0.248	C3 > C0
Mm	August	8.12	2	0.017	C1 > C2 > C0
	September	4.08	2	0.130	C2 > C3 > C0
	October	0.01	1	0.913	C1 > C0
Mn	August	3.29	1	0.070	C1 > C0
	September	33.23	3	<0.0001	C1 > C2 > C3 > C0
	October	33.38	2	<0.0001	C3 > C2 > C0

[1] Mbr = *M. brandtii,* Md = *M. daubentonii*, Mm = *M. mystacinus*, Mn = *M. nattereri*
[2] C0 = Condition 0, C1 = Condition 1, C2 = Condition 2, C3 = Condition 3.

In all cases bats in Condition 0 had lowest median body condition (Table 6.3). In *M. brandtii, M. daubentonii* and *M. mystacinus* there was a significant difference in body condition among bats in the different reproductive conditions in August. Bats undergoing spermatogenesis (Condition 1) or in the sperm storage phase (Condition 3) had better body condition than bats in Condition 0. In September and October, despite there being no statistical difference in mean body condition between the reproductive categories in *M. daubentonii* and *M. mystacinus* there was a trend for better body condition in Conditions 2 and 3 than in Condition 0. Body condition of *M. nattereri* was significantly different at different reproductive stages in September and October. In September those in Conditions 1 and 2 were of best body condition, but in October bats in Conditions 3 and 2 were of best condition. This would be expected from continued development of the heaviest and most reproductively advanced individuals from month to month.

In three out of four of the species tested there was no significant difference between adults and juveniles in forearm length (Table 6.4) indicating that juveniles had already reached adult body size by the swarming season but not adult body mass.

Table 6.4. Results of Mann-Whitney U tests testing for differences in forearm length between adult and juvenile males of four species[1].

Species	Median forearm (mm)	N_1 N_2	W	P
Mbr Adult	34.7	26	484.5	0.490
Mbr Juvenile	34.9	12		
Md Adult	37.1	258	48459.5	0.002
Md Juvenile	36.8	96		
Mm Adult	33.7	71	4591.0	0.824
Mm Juvenile	33.7	56		
Mn Adult	39.0	599	213865.5	0.157
Mn Juvenile	38.9	105		

[1] Mbr = *M. brandtii*, Md = *M. daubentonii*, Mm = *M. mystacinus*, Mn = *M. nattereri*

Further analysis on forearm lengths of male *M. daubentonii* showed that during August and September there was no difference between forearm lengths of adults and juveniles (W = 7485, N_1 = 109, N_2 = 23, P = 0.157; W = 96465, N_1 = 111, N_2 = 55, P = 0.195) but during October average forearm length was shorter in juveniles than in adults (W = 462.5, N_1 = 22, N_2 = 13, P = 0.024) however this could be an artifact of the smaller sample size during October.

The effect of body condition on sexual development could also be followed in those individuals that were ringed and recaptured at a later date. Occasions of bats being caught on close enough dates for comparison are few hence no statistics have been presented here, only observations, however they support the finding that early onset or delay of development is connected with body condition. The *M. daubentonii* caught on 12 August 2001, when it had more advanced development of the reproductive organs (Condition 2), had better body condition (+0.796) than on 16 August 2000 (-0.115; Condition 1). Another *M. daubentonii* caught on the same two occasions showed the converse. It had less developed sexual organs (Condition 1) on 12 August 2001 and poorer body condition (-0.014) than on 16 August 2000 (+0.221; Condition 2). The same pattern was seen in recaptured *M. nattereri*. A bat caught on 16 September 2001 was in a more advanced reproductive condition (Condition 2) and in better body condition (+0.200) than on 22 September 1999 (-1.561; Condition 1).

6.3.6. Reproductive and body condition in females

Female *M. daubentonii* and *M. nattereri* classed as parous adults had better body condition on average than those classed as non-parous or juveniles, but there was no difference in forearm length between the two groups (Table 6.5). Sample sizes for females of other species were insufficient for analysis.

Table 6.5. Results of Mann-Whitney U tests testing for differences in relative body condition (BCI) and in forearm length between adult (parous) females and nulli-parous or juvenile females.

Species	Median relative BCI (g)	N_1 N_2	W	P
Md Adult	+0.033	83	2834	0.002
Md Juvenile	-0.634	52		
Mn Adult	-0.083	226	12465	0.001
Mn Juvenile	-0.356	93		
Species	**Median forearm (mm)**	N_1 N_2	**W**	**P**
Md Adult	37.45	82	5111	0.943
Md Juvenile	37.40	42		
Mn Adult	39.80	199	27094.5	0.077
Mn Juvenile	39.50	63		

Md = *M. daubentonii*, Mn = *M. nattereri*

6.3.7. Body masses and forearm lengths in males and females

Mass gain in females during the swarming season closely mirrored that of males (Fig. 6.6). Actual masses have been used rather than body condition indices to visualize the difference in body mass between males and females and between species. Sample size was too small and the variance too great in *M. bechsteinii* to see a trend. Female *M. mystacinus* were heavier on average than males throughout the season and the pattern of mass increase was similar for both sexes, but occurred earlier in females than in males. Males and females of *M. brandtii, M. daubentonii, M. nattereri* and *P. auritus* were more similar in mass, especially early in the season. *M. nattereri* females showed greater increase in body mass than males in October and November. The greatest change in body mass during the course of the year was seen in both sexes of *M. daubentonii* (♂ 53% and ♀ 80% increase on lowest mean mass). For the remaining species the increase was in the order of 10 to 40% of lowest mean body mass. There was a trend for females to have a greater range in body mass than males during the course of the year (in 4 out of 6 species). Females had significantly longer forearms than males in five out of the six species (Table 6.6). In *M. brandtii* there was no significant difference between the sexes in forearm length.

Table 6.6. Results of Mann-Whitney U tests testing for differences in forearm length between males and females of each species[1].

Species and sex	Median forearm (mm)	N_1 N_2	W	P
Mbe ♂	40.7	88	4182.5	0.016
Mbe ♀	41.6	11		
Mbr ♂	34.9	100	9170.5	0.149
Mbr ♀	34.6	73		
Md ♂	37.0	445	124606.5	0.0001
Md ♀	37.4	146		
Mm ♂	33.7	176	18764	<0.00001
Mm ♀	34.4	61		
Mn ♂	39.0	835	440438	<0.00001
Mn ♀	39.8	329		
Pa ♂	38.0	80	3609	0.008
Pa ♀	38.8	16		

[1] Mbe = *M. bechsteinii*, Mbr = *M. brandtii*, Md = *M. daubentonii*, Mm = *M. mystacinus*, Mn = *M. nattereri*, Pa = *P. auritus*

Figure 6.2. Change in condition of testes and epididymides during part of the reproductive cycle in *M. bechsteinii*, *M. brandtii*, *M. daubentonii*, *M. mystacinus*, *M. nattereri* and *P. auritus*. Sample size is given above each bar. Note *x*-axis begins at July.

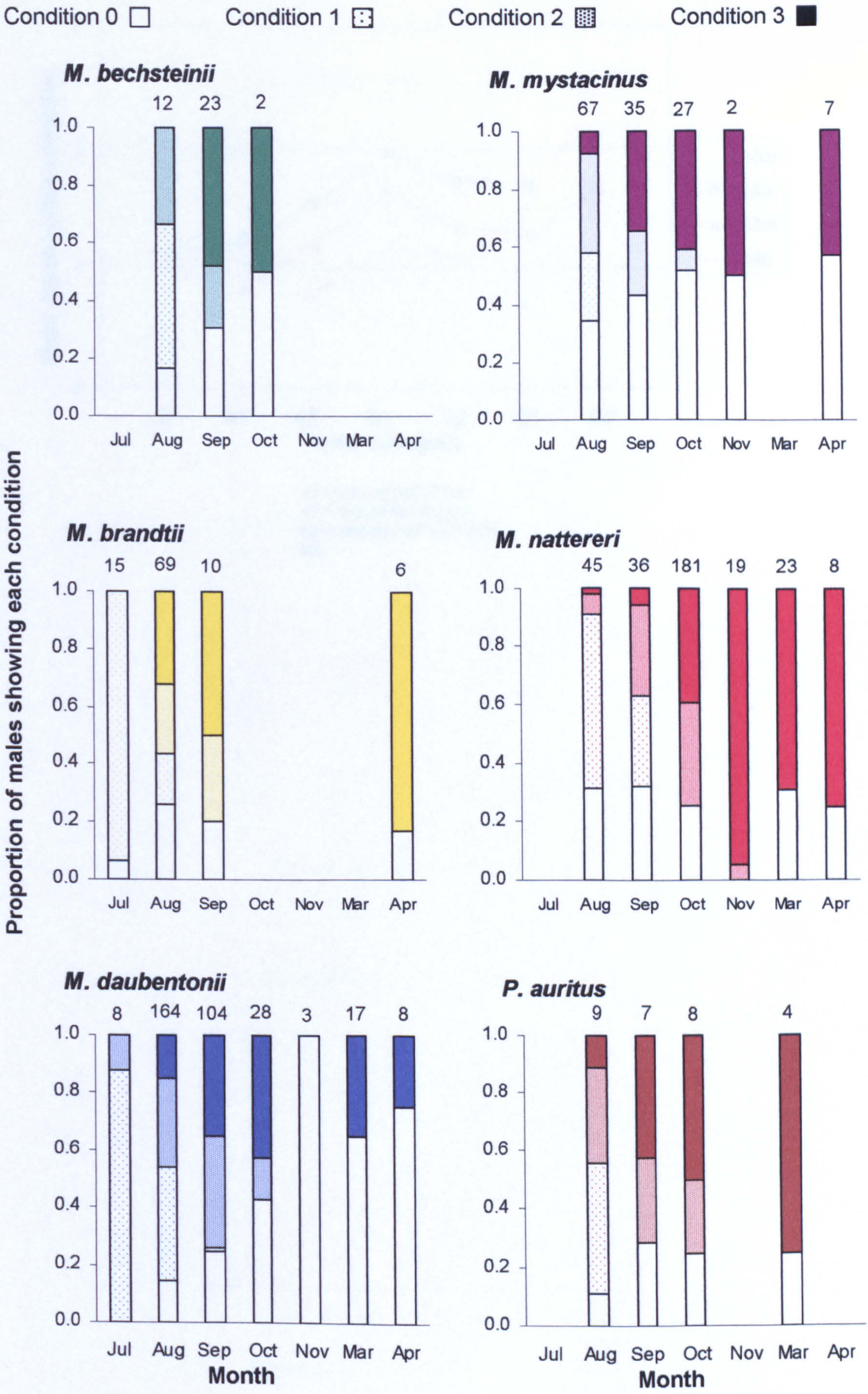
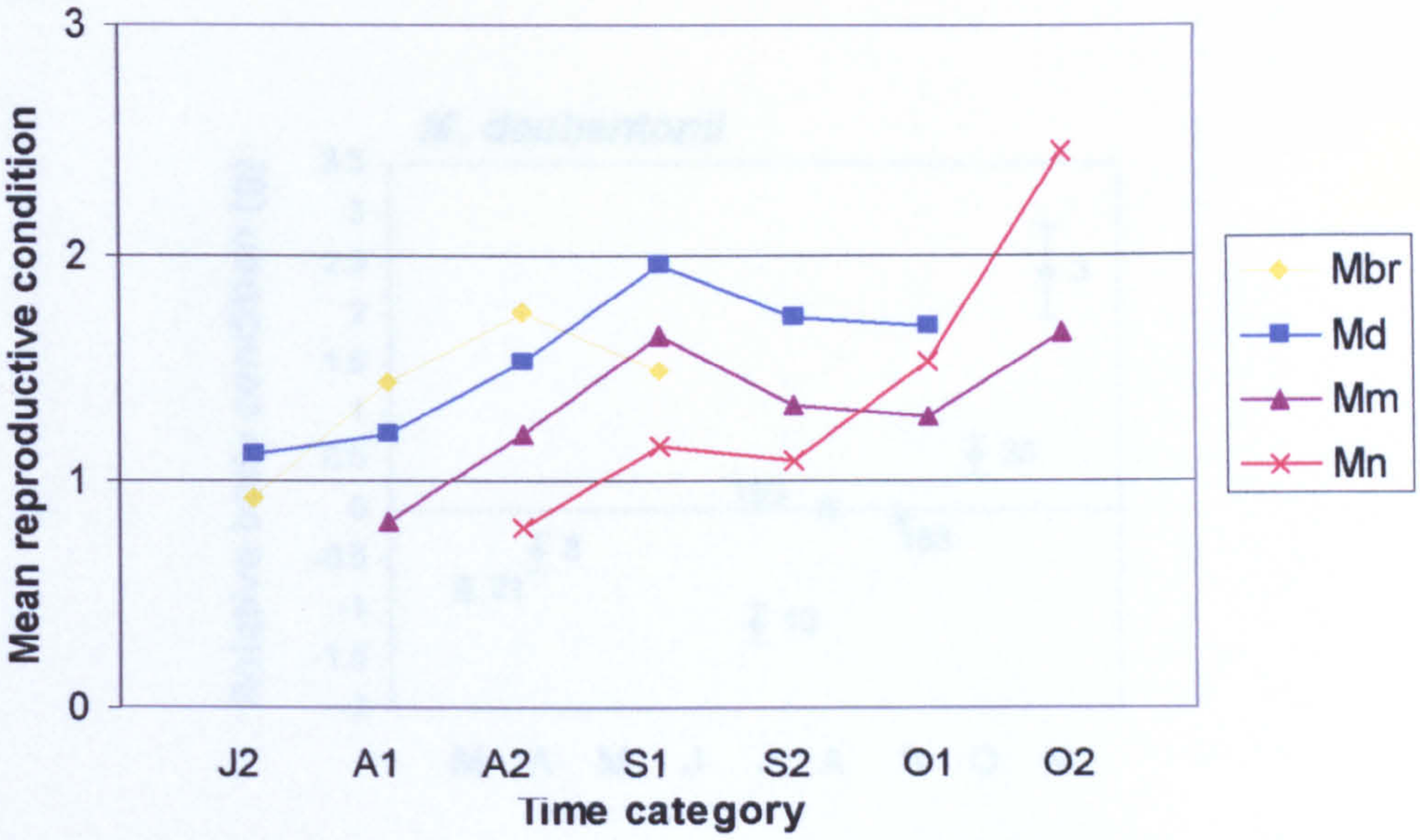


Figure 6.3. Mean reproductive condition of male *M. brandtii*, *M. daubentonii*, *M. mystacinus* and *M. nattereri* at half-monthly intervals during the swarming season.



J2 = second half of July
A1 = first half of August
A2 = second half of August
etc.

Figure 6.4. Mean relative body condition of male bats of three species (mean ± SE) monthly from March to November. Sample size is given next to each data point. Body condition values above the zero line are heavier than expected and values below the zero line are lighter.

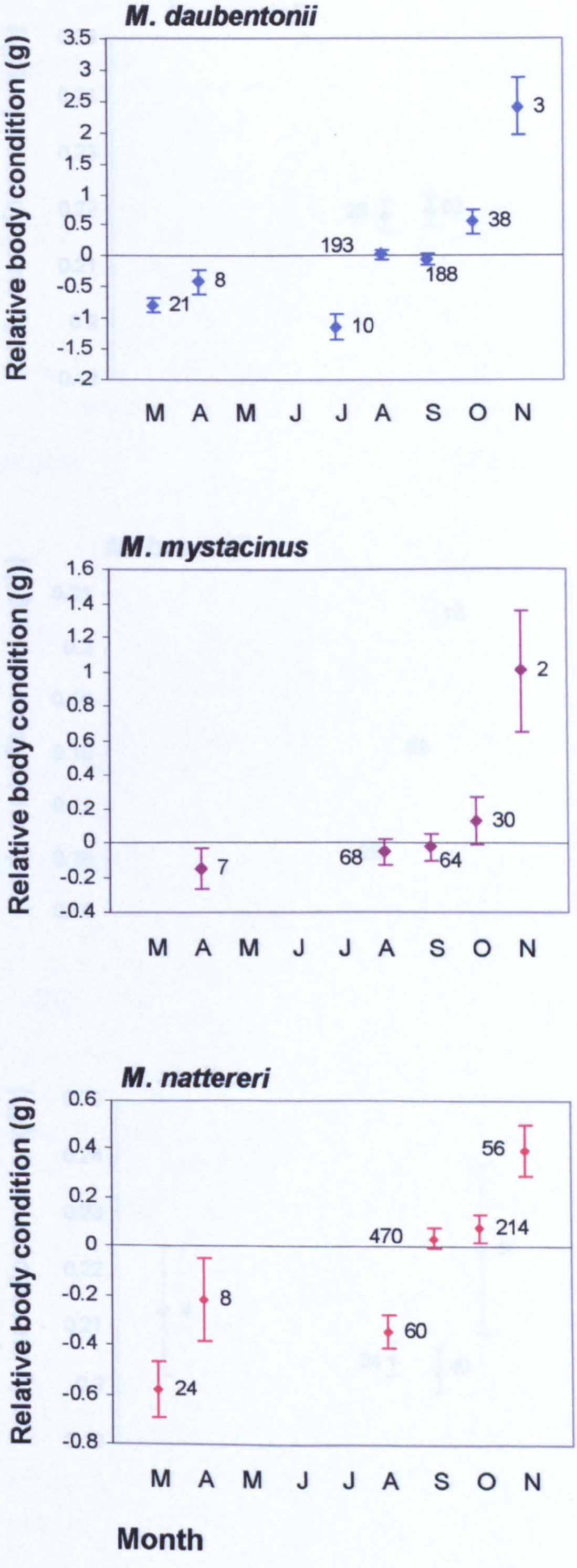


Figure 6.5. Mean (± SE) actual body condition of male bats of three species monthly from March to November. Sample size is given next to each data point.

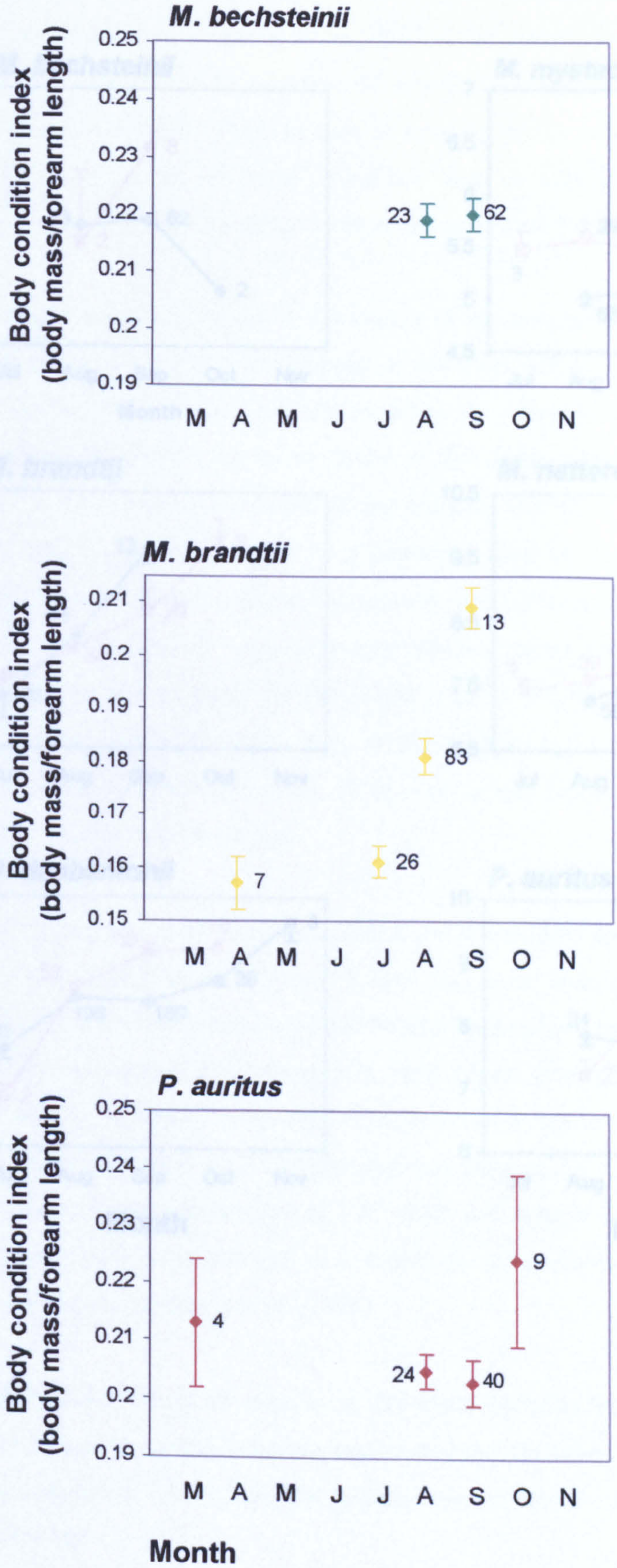
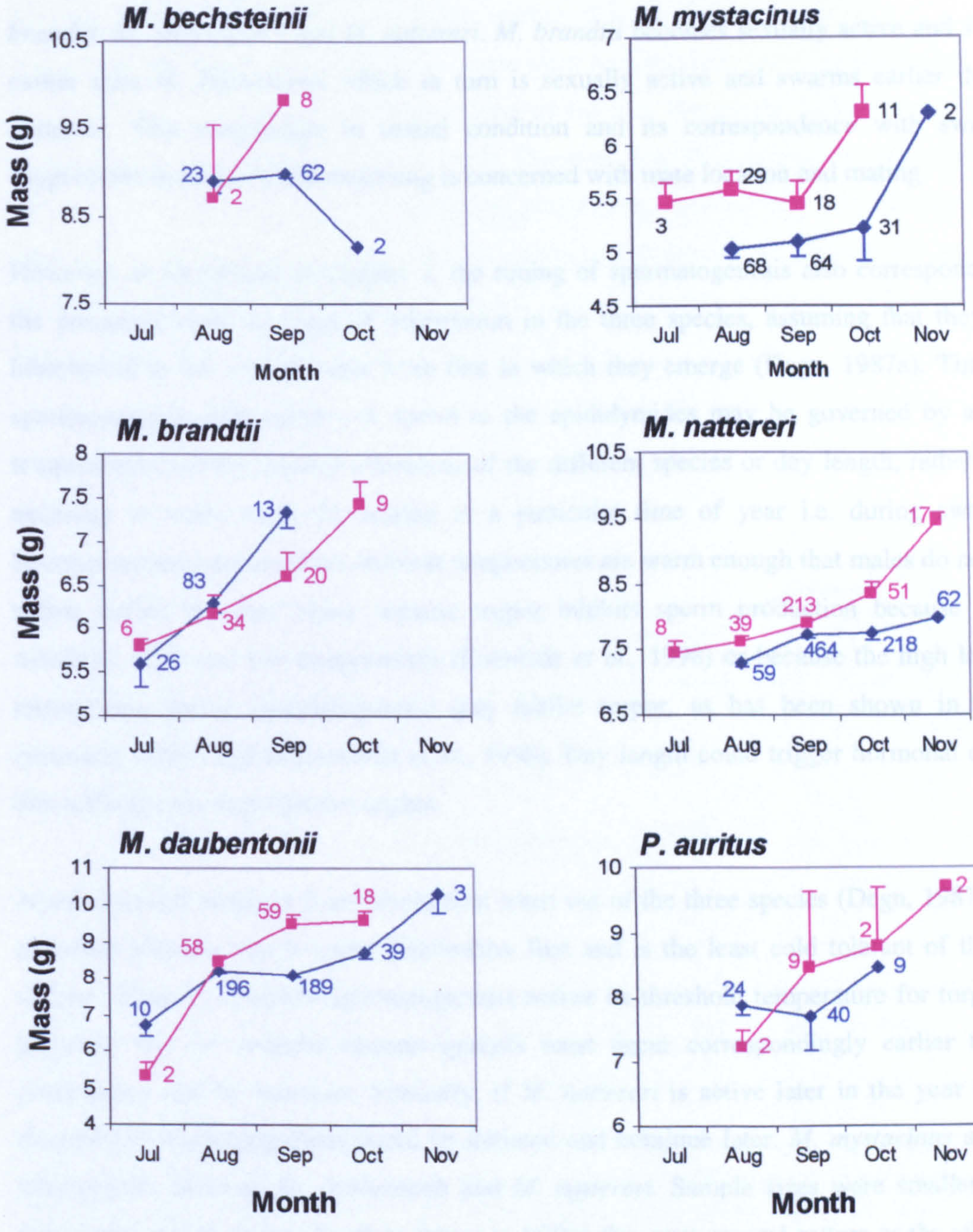


Figure 6.6. Average body masses of males and females of six species (mean ± SE) monthly from July to November. Sample sizes are given next to each data point.



6.4. DISCUSSION

6.4.1. Reproductive timing in male bats

The pattern of timing of spermatogenesis and onset of sperm storage in the different species corresponded with their order of dominance at the swarming site during swarming (see Figs. 2.7 and 2.8 in Chapter 2) (Parsons *et al.*, 2003). The pattern is most marked among *M. brandtii, M. daubentonii* and *M. nattereri. M. brandtii* becomes sexually active and swarms earlier than *M. daubentonii* which in turn is sexually active and swarms earlier than *M. nattereri.* This progression in sexual condition and its correspondence with swarming supports the hypothesis that swarming is concerned with mate location and mating.

However, as mentioned in Chapter 2, the timing of spermatogenesis also corresponds with the presumed order of onset of hibernation in the three species, assuming that they enter hibernation in the reverse order from that in which they emerge (Degn, 1987a). Timing of spermatogenesis and transfer of sperm to the epididymides may be governed by ambient temperatures and the thermal tolerances of the different species or day length, rather than a necessity to ready them for mating at a particular time of year i.e. during swarming. Spermatogenesis occurs when ambient temperatures are warm enough that males do not enter torpor during the day, either because torpor inhibits sperm production because of low metabolic rates and low temperatures (Entwistle *et al.*, 1998) or because the high levels of testosterone during spermatogenesis may inhibit torpor, as has been shown in rodents (Bronson, 1989 cited in Entwistle *et al.*, 1998). Day length could trigger hormonal changes that influence the reproductive organs.

Myotis brandtii emerges from hibernation latest out of the three species (Degn, 1987a). It is therefore possible that it enters hibernation first and is the least cold tolerant of the three species. Thus, to complete spermatogenesis before its threshold temperature for torpor it is plausible that *M. brandtii* spermatogenesis must occur correspondingly earlier than *M. daubentonii* and *M. nattereri.* Similarly, if *M. nattereri* is active later in the year than *M. daubentonii* spermatogenesis could be initiated and continue later. *M. mystacinus* appeared intermediate between *M. daubentonii* and *M. nattereri.* Sample sizes were smaller for *M. bechsteinii* and *P. auritus* but they appear to follow the same general pattern as the others and support the findings of Entwistle *et al.* (1998).

There are few observations of copulation for these species. Stebbings (1966) observed copulation in *P. auritus* in mid-September and noted that the testes regressed during October and were not visible in November. My findings suggest that *P. auritus* would be ready to mate by September.

Because of the facility to store sperm, bats that are ready for mating may not necessarily mate immediately. Figure 6.7 displays data collected by Strelkov (1960) on incidence of insemination in females of three of the species I studied. The date at which 50% of the bats sampled had been inseminated varied among the species. The earliest was *M. daubentonii* at the end of October, then *P. auritus* in mid-December, followed by *M. mystacinus* in March. This figure is not consistent with prolific mating of females during swarming at Strelkov's site, except perhaps in *M. daubentonii*, where most females were mated by November. Some *M. daubentonii* were observed mating during winter even though the main period of mating in this species is in October (Harrje, 1994). However, few *M. daubentonii* were captured at swarming sites after the end of September, presumably because they had already entered hibernation. Mating in all three species obviously continues thoughout the period of hibernation (Strelkov, 1960). *M. nattereri* was observed mating at the end of December in a hibernaculum (Gilbert & Stebbings, 1958).

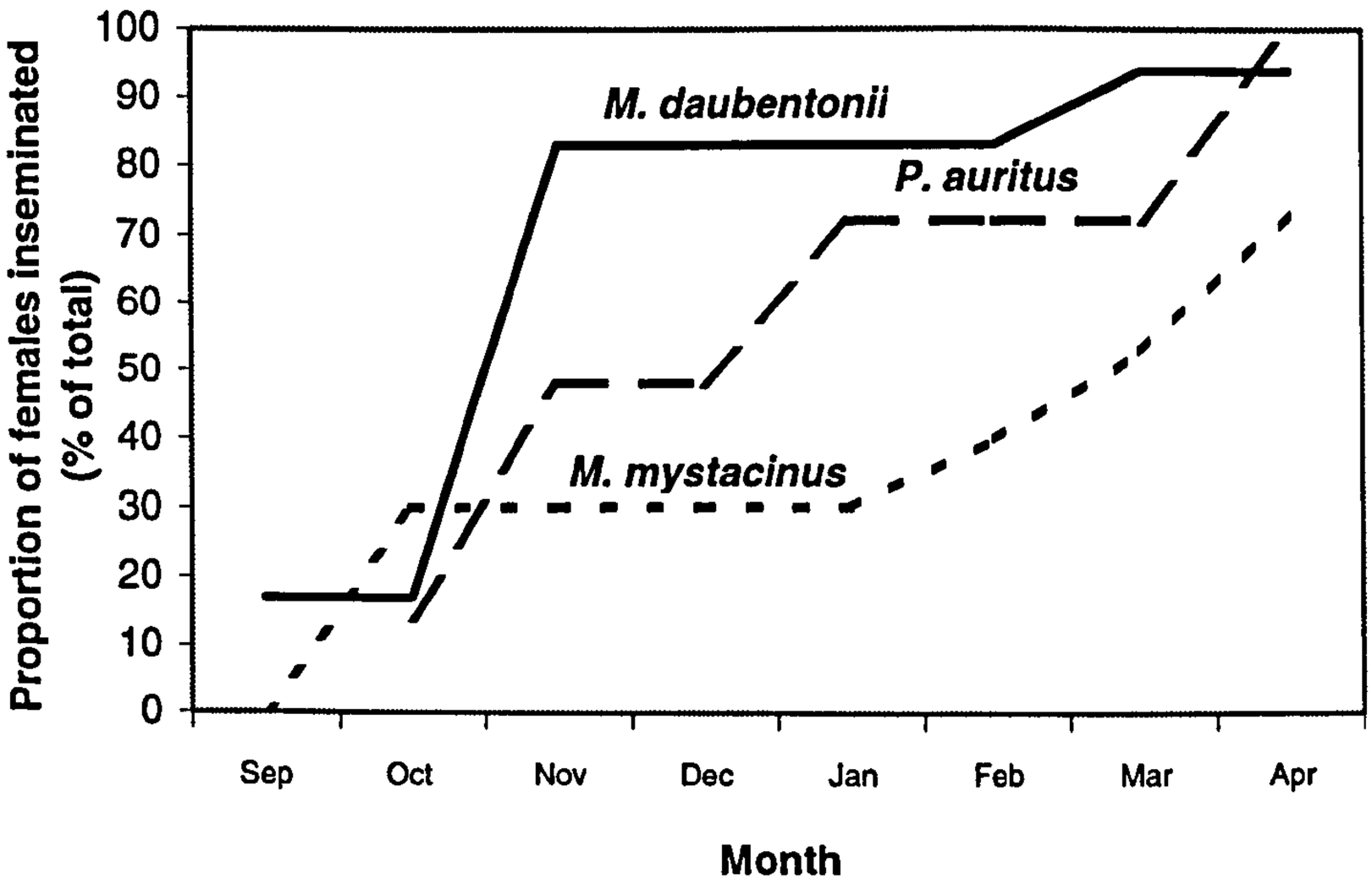


Figure 6.7. Chart constructed from data in Strelkov (1960) showing proportion of females found to be inseminated from those captured during pre-hibernation (October/November) flights, during hibernation, and during dispersal from hibernation (April) for three species of vespertilionid bat.

Racey *et al.* (1987) found that the proportion of female *M. lucifugus* inseminated at a swarming site did not increase during swarming, suggesting either that females left the area after insemination or perhaps that many did not mate until after the end of observation, which was in early September.

Some males still had distended epididymides in March and April indicating that sperm were available for potential spring mating. Sperm stored for up to seven months in male *Nyctalus noctula* still produced viable offspring (Racey, 1973; Racey, 1979). Stebbings (1966) found that the testes of *P. auritus* had begun to enlarge as early as 8 April however no *P. auritus* were caught in April in this study and enlarged testes were not seen in any of the *Myotis* spp. in April.

6.4.2. Onset of sexual maturity in males

At all survey times, some individuals were caught with no testicular or epididymal distension. A large proportion of these individuals were identified as young-of-the-year. The results suggest that an influx of immature males occurs in the latter part of the swarming season. Perhaps they are investigating hibernation sites or beginning to arrive at the site for hibernation. Accurate separation of adults from juveniles is problematic during the autumn (Thomas *et al.*, 1979) thus I cannot have complete confidence in my results. However, I found that in *M. mystacinus* the phalangeal joints in the wing of a juvenile remained tapered and distinguishable from that of an adult into October. Therefore, I had greater confidence in ageing this species than for other species. I conclude that perhaps as many as 30-40% of juveniles become sexually mature in their first season. The percentage of young becoming sexually mature (exhibiting conditions 1, 2 or 3) was highest for *M. daubentonii, M. brandtii* and *M. mystacinus* but was smaller for *M. bechsteinii* and *M. nattereri*.

A relationship may exist between size of the bat and time of sexual maturity in these species with the smaller species becoming morphologically and sexually mature in a shorter time than the larger species. Rank size of species from smallest to largest mean forearm is *M. mystacinus* < *M. brandtii* < *M. daubentonii* < *P. auritus* < *M. nattereri* < *M. bechsteinii.* A similar relationship between size and onset of sexual maturity is seen for *R. hipposideros* and *R. ferrumequinum* where the former smaller species can become sexually mature in the spring following its birth but the latter become mature only at two, three or even four years of age (Stebbings, 1988). Similarly, juvenile male *M. lucifugus* in Alberta, Canada showed no sign of reproductive activity in their reproductive organs, but most juvenile *M. volans* caught at the same time were reproductively active (Schowalter, 1980). *M. volans* is the smaller of the two species. My estimate for the proportion of males becoming mature in their first season is similar to that of Entwistle *et al.* (1998) for *P. auritus* and lends further support to a statement by Stebbings (1988) that in northern Europe some bats breed in their first year, but the majority breed in their second year. Kokurewicz and Bartmańska (1992) also found that male *M. daubentonii* attained sexual maturity within three to four months of birth.

6.4.3. Pigmentation of the tunica vaginalis

Some individuals known to be greater than one year old still had a pigmented or partially pigmented tunica vaginalis covering the epididymis, consistent with findings of Entwistle *et al.* (1998) for *P. auritus* and Kokurewicz and Bartmańska (1992) for *M. daubentonii.* In *M. mystacinus* in particular the epididymides often appeared speckled or grey in adults. In other species the epididymides were often totally white or white with a black tip, presumably because they were filled with spermatozoa, but the following year prior to sperm transfer the epididymides were darkly pigmented again. Presumably the pigment is either lost entirely during sperm storage and then synthesized anew the following year or is pushed to the most distal region of the epididymis by the increasing volume of spermatozoa and becomes redistributed once the sperm is removed. Dispersion of the melanin pigment is certainly not a permanent condition in these species. I consider that in none of the species studied would the pigmentation of the tunica vaginalis be a reliable indicator of sexual maturity as shown for *P. pipistrellus* (Racey, 1974a).

6.4.4. Male reproductive and body condition

Body masses recorded for each of the species were within the ranges published previously (Appendix 4). All males had poor body condition in March and April as expected from use of body fat reserves during winter. Patterns of body mass gain prior to hibernation are consistent with *M. brandtii* entering hibernation early, followed by *M. daubentonii*, *M. mystacinus* and *M. nattereri.*

It has been suggested that males lose body mass during the period of sexual activity and increase body mass quickly before hibernation (Lundberg & Gerell, 1986). Hendricks *et al.* (2000) found that both male and female *E. fuscus* weighed less in October than in September and Schowalter (1980) found that male *M. lucifugus* lost body mass before hibernation but *M. volans* did not. Consistent with Entwistle *et al.* (1998) male *P. auritus* in our study lost mass between August and September, then increased by October. *M. daubentonii* had low body mass in July, and *M. nattereri* in August, compared to other months. Encarnação *et al.* (2002) did not report a reduction in body mass of *M. daubentonii* in their study during summer.

Perhaps energetic investment in spermatogenesis causes mass loss, however, if this were the case mass loss in *P. auritus* is delayed compared to its time of peak spermatogenesis. Instead mass loss might result from increased flight activity during swarming rather than the energetic demands of spermatogenesis. Lundberg and Gerell (1986) suggested that the decrease in body mass of male *Pipistrellus pipistrellus* (probably *P. pygmaeus*) during mating was due to the energetic demands of song-flight displays used to attract females.

Although the analyses testing for relationships between body condition and reproductive condition were not conclusive for each species some interesting trends emerged. In *M. daubentonii* and *M. brandtii* (in August) and *M. nattereri* (in September) bats undergoing spermatogenesis were heavier than bats with no obvious testicular development. Bats with no testicular development were probably either young of the year, hence lighter and less likely to become sexually mature in their first year, or adults with poor body condition, which may have delayed spermatogenesis. Kunz *et al.* (1998) found that young *M. lucifugus* had approximately 20% lower lean body mass and fat index than adults during swarming and Davis and Hitchcock (1965) and Schowalter (1980) found that juvenile *M. lucifugus* accumulated fat more slowly and weighed less than adults upon entering hibernation.

Later in the season (September for *M. daubentonii* and *M. mystacinus,* and October for *M. nattereri*), the heaviest bats were those in the sperm transfer or sperm storage phases, consistent with the ongoing development of the heaviest individuals from the previous months. These results are consistent with the heaviest bats, those in best condition, undergoing spermatogenesis and the lightest bats (many probably young of the year) remaining in a quiescent state with no sexual development. Recaptures of ringed bats demonstrated that body condition in a particular year can delay or advance development of the reproductive organs.

With the exception of *M. nattereri,* there was no significant difference in body condition among bats exhibiting the different reproductive categories in the latter months of the swarming season, indicating that the lighter individuals had gained proportionately more body mass before hibernation than their heavier counterparts and body condition was more similar across the reproductive conditions.

Difficulty exists in separating cause and effect in the relationship between body condition and reproductive condition. It has been assumed in past studies that the heavier bats develop earlier or more completely than lighter bats (Entwistle *et al.,* 1998; Speakman & Racey, 1986). However, the increased body mass (better body condition) recorded by researchers could be attributable to the combined mass of the testicular tissues and the stored spermatozoa and hence be a consequence of the reproductive activity rather than the cause of it. In extreme cases bat testes can increase in mass during spermatogenesis by up to 40 times (Racey & Tam, 1974).

Wai-Ping and Fenton (1988) found that female *M. lucifugus* did not selectively mate with the heaviest males available so it would appear that although body condition might influence the

timing of development of the sexual organs it does not influence the chances of a male mating successfully.

6.4.5. Female reproductive and body condition

Consistent with the findings for males, female young of the year were in poorer body condition than adult parous females despite having similar forearm lengths. Juvenile bats rapidly attain adult forearm length during the summer of their birth, but require longer to attain adult body mass therefore are in poorer body condition during swarming than adults. Female bats followed a broadly similar pattern of body mass increase before hibernation to that of males.

In conclusion, the timing of annual development of the male sexual organs occurs synchronously with the time of peak swarming in vespertilionid bats. This suggests that reproductive development is closely allied to swarming activity for purposes of mating, or that reproductive development is closely allied to onset of hibernation, which is preceded by swarming. Males in better body condition are likely to be in a more advanced state of reproductive development than those in poor body condition.

Possible future work in this area could be to discover whether females visiting a site during swarming are actually mating. This could be achieved by douching the vagina of a female bat with saline solution and preparing a slide of the sample. This could then be viewed with a microscope in the laboratory for the presence of spermatozoa, which would confirm that the female had been mating that night. Further, more detailed investigations into the hormonal and environmental triggers for the annual development and regression of the male sexual organs would be beneficial to understanding the male sexual cycle, in particular why differences in timing exist between the different species.

CHAPTER SEVEN

GENETIC VARIATION IN *M. NATTERERI* AT SWARMING SITES AND AT MATERNITY COLONIES

7. GENETIC VARIATION IN *M. NATTERERI* AT SWARMING SITES AND AT MATERNITY COLONIES

SUMMARY

This preliminary study of the genetics of *M. nattereri* investigated population substructure at two swarming sites and two maternity colonies in southwest England by using microsatellite DNA markers.

It was predicted that if mating predominantly occurs at swarming sites genetic variation will be great, both at the swarming sites and at maternity colonies within their catchment area, and evidence for either random mating or an excess of heterozygotes (indicative of out-breeding) should be found. If, however mating occurs at the colony level and not at the swarming sites, it is predicted that genetic variation among bats gathered at swarming sites will be very much greater than variation among bats in individual colonies, and that colonies will be genetically differentiated from one another. Genetic differentiation might increase with geographical distance between colonies.

Allelic diversity was high at both swarming sites and maternity colonies. Departures from Hardy-Weinberg equilibrium were found for males at swarming sites, indicating that there may be active out-breeding. There was no significant genetic differentiation between the different colonies and the swarming sites, indicating that there is little population substructure and that mating and consequently gene flow occurs between all groups in the area. The evidence suggests that mating in *M. nattereri* occurs at swarming sites rather than at the colony level, supporting the theory that swarming facilitates the location of mates from a normally widely dispersed population, and consequently promotes gene flow in that population.

Further work incorporating more distant colonies and swarming sites, and to determine whether female natal philopatry and male dispersal operate is required to further investigate the mechanisms of gene flow in *M. nattereri.*

7.1. INTRODUCTION

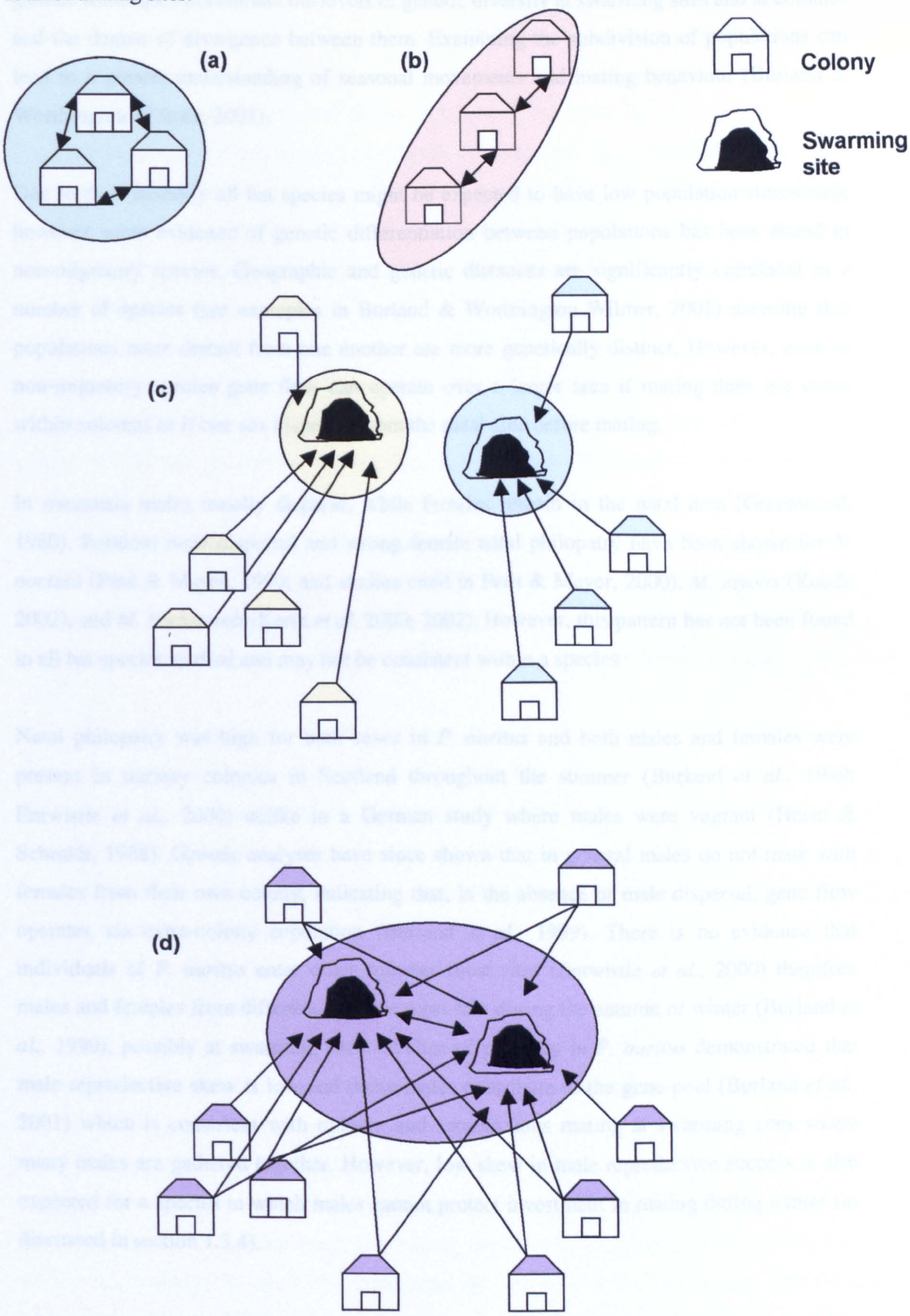
In Chapters 2 and 4, I showed that swarming sites are visited by thousands of *M. nattereri* during the swarming season each year. These bats are drawn from wide catchment areas containing numerous colonies (Chapter 5). A colony can be defined as a group of individuals that interact with one another to a distinctly greater degree than with other conspecifics (Burland & Worthington Wilmer, 2001). In summer, female *M. nattereri* gather at traditional roosts in trees or buildings. Maternity roosts, where they give birth to their young, are most often in the attics of old stone barns or houses (Smith & Racey, 2002). Male *M. nattereri* are probably dispersed in the environment at this time. Park *et al.* (1998) showed that *M. nattereri* form mixed-sex groups in bat boxes during the mating season but whether mating occurs within these associations, between adjacent colonies or elsewhere is not known.

7.1.1. Hypotheses of gene flow

Marking studies have shown that bats from different colonies gather together at seasonal refuges for autumnal swarming (Chapter 5) and/or hibernation (Barbour & Davis, 1969; Davis & Hitchcock, 1965). Petit and Mayer (2000) surveyed mitochondrial DNA sequences in *N. noctula* and showed that there was considerably higher diversity among individuals in hibernacula, confirming that they came from different nursery colonies.

Humphrey and Cope (1976) suggested that autumnal swarming might be an important aspect of gene flow, because it may be the mechanism by which individuals dispersed over a wide area come together to breed, and failure to find mates and local inbreeding are thus prevented. This theory is supported by observations of copulations during swarming (Racey *et al.*, 1987; Thomas *et al.*, 1979) and by the mature sexual condition of swarming males (Chapter 6). If swarming sites do function as mating sites for bats from a wide area (4118 km^2 – Chapter 5) then gene flow would occur between many colonies and a high degree of genetic variation would be expected in all colonies and in the swarming population (Fig. 7.1d). There would be little between-colony differentiation. However, if bats are faithful to one particular swarming site, populations visiting each swarming site might become genetically distinct over time (Fig. 7.1c). But if mating occurs at the colony level or between adjacent colonies (Fig. 7.1a & b), perhaps in mating groups established near to maternity roosts, widely-spaced colonies should become increasingly genetically differentiated and the degree of genetic variation at swarming sites should be greater than that in each colony. The possible mechanisms of gene flow and population structures depending on where mating occurs are shown in Figure 7.1.

Figure 7.1. Four population structures depending on where mating takes place: (a) between adjacent colonies; (b) between neighbouring colonies in a linear stepwise fashion; (c) between colony groups at different swarming sites; and (d) between all colonies at all swarming sites.



7.1.2. Previous genetic studies on bats

The hypotheses concerning where mating takes place can be tested by using molecular genetic techniques to examine the levels of genetic diversity at swarming sites and at colonies and the degree of divergence between them. Examining the subdivision of populations can lead to a greater understanding of seasonal movements and mating behaviour (Burland & Worthington Wilmer, 2001).

Due to their mobility all bat species might be expected to have low population structuring; however some evidence of genetic differentiation between populations has been found in non-migratory species. Geographic and genetic distances are significantly correlated in a number of species (see examples in Burland & Worthington Wilmer, 2001) meaning that populations more distant from one another are more genetically distinct. However, even in non-migratory species gene flow can operate over a larger area if mating does not occur within colonies or if one sex disperses from the natal area before mating.

In mammals males usually disperse, while females remain in the natal area (Greenwood, 1980). Random male dispersal and strong female natal philopatry have been shown for *N. noctula* (Petit & Mayer, 1999; and studies cited in Petit & Mayer, 2000), *M. myotis* (Ruedi, 2002), and *M. bechsteinii* (Kerth *et al.* 2000; 2002). However, this pattern has not been found in all bat species studied and may not be consistent within a species.

Natal philopatry was high for both sexes in *P. auritus* and both males and females were present in nursery colonies in Scotland throughout the summer (Burland *et al.*, 1999; Entwistle *et al.*, 2000) unlike in a German study where males were vagrant (Heise & Schmidt, 1988). Genetic analyses have since shown that in general males do not mate with females from their own colony, indicating that, in the absence of male dispersal, gene flow operates via extra-colony copulation (Burland *et al.*, 1999). There is no evidence that individuals of *P. auritus* enter other summer roost sites (Entwistle *et al.*, 2000) therefore males and females from different colonies must mix during the autumn or winter (Burland *et al.*, 1999), possibly at swarming sites. Studies of paternity in *P. auritus* demonstrated that male reproductive skew is low and many males contribute to the gene pool (Burland *et al.*, 2001) which is consistent with random and promiscuous mating at swarming sites where many males are gathered together. However, low skew in male reproductive success is also expected for a species in which males cannot protect investment in mating during winter (as discussed in section 1.3.4).

Colonies of *P. auritus* in three regions of Scotland formed a continuously distributed population, within which genes moved via a 'stepping stone' model (Burland *et al.*, 1999) (Fig. 7.1b). This suggests that rather than mating at swarming sites, where colony members gather at a central location, *P. auritus* mates with bats from the colony (or colonies) adjacent to their own. Alternatively bats might visit the swarming sites nearest to them and therefore overlap with adjacent but not more distant colonies. If this were the case the population's genetic structure would consequently be likely to depend on the availability of swarming sites in the locality. It is of course possible that two mating strategies are adopted by males, firstly maintaining a harem of females at a mating roost and additionally visiting swarming sites to obtain extra copulations. In this case the information gleaned from a genetic study is likely to be less clear-cut.

In *M. lucifugus*, the best-researched swarming species in North America, evidence of male genetic skew has been found (Watt & Fenton, 1995) suggesting that discriminate mating occurs, or that post-copulatory sperm competition operates. Male genetic skew could still occur when mating takes place at swarming sites, however the presence of female choice and male advertisement or competition has not been adequately proven in this species (Racey *et al.*, 1987; Sparks *et al.*, 2000; Thomas *et al.*, 1979). The presence of reproductive skew is not expected in a species that is unable to protect investment in mating. Perhaps matings during winter are ineffectual or post-copulatory sperm competition operates to favour certain males.

A recent study (Kerth *et al.*, 2003) used mitochondrial microsatellite DNA markers to compare the gene diversity of *M. bechsteinii* at breeding colonies and at potential mating sites (swarming sites), a technique similar to that used in my study. It was found that swarming sites had greater mitochondrial gene diversity than colonies indicating that swarming sites provide the opportunity for gene flow among bats originating from different colonies (Kerth *et al.*, 2003).

7.1.3. Molecular genetic techniques

The use of molecular genetic techniques is an invaluable resource in studying bats, which is often difficult by direct observation (Burland & Worthington Wilmer, 2001), particularly during the mating season in some temperate species. Microsatellite DNA markers have been widely used and are particularly beneficial because non-lethal sampling can be used and small amounts of DNA are amplified through polymerase chain reaction (PCR) for analysis. Microsatellites are simple sequences of tandemly repetitive DNA, between one and five base pairs long (Campbell *et al.*, 1999), present in the genomes of most organisms (Hancock, 1999; Tautz & Renz, 1984). Units are repeated only 10 to 100 times (Campbell *et al.*, 1999).

They are usually highly polymorphic and are predominantly selectively neutral (Hancock, 1999). Microsatellite markers have already been isolated and characterized for several vespertilionid bat species and are available for use in this study. Burland *et al.* (1998) showed that alleles were obtained at four out of six loci isolated for *P. auritus* in cross-amplification with *M. nattereri.*

To investigate the degree of genetic differentiation between populations microsatellite markers are used to determine the frequency of different alleles for a number of loci in the genomes of different populations. Where there are no perturbing forces (such as mutation, migration or selection) allele and genotype frequencies attain equilibrium after one generation of random mating in a large population (Frankham *et al.*, 2002), known as the Hardy-Weinberg equilibrium. Tests for deviation from Hardy-Weinberg equilibrium may reveal whether non-random mating, migration or selection has occurred.

Allele frequencies are used to calculate F_{ST}, a measure of the correlation of genes of different individuals in the same subpopulation (Weir & Cockerham, 1984). If the heterozygosity observed in the subpopulation differs from that in the total population, then that sub-population can be considered differentiated. The level of genetic similarity or differentiation between different populations can be assessed by pair-wise comparisons of F_{ST} of those populations (Burland & Worthington Wilmer, 2001). Exact tests of allelic and genotypic differentiation are also possible. Isolation by distance among colonies can be assessed by a matrix correlation analysis (Mantel test) for the presence of a significant association between genetic and geographical distances (Burland & Worthington Wilmer, 2001). Also of interest might be F_{IT}, which estimates the correlation of genes within an individual (inbreeding) compared to the overall population and F_{IS}, which estimates the correlation of genes within individuals within a subpopulation (Weir & Cockerham, 1984).

The specific aims of this chapter are:

1. To study the population structure of *M. nattereri* in the catchment area of two swarming sites in south-west England by micro-satellite DNA analysis.
2. To determine the level of genetic variation within subpopulations at swarming sites and at maternity colonies within the catchment and to assess whether the subpopulations are genetically different from one another.
3. To determine whether members of two maternity colonies, separated by 36 km are genetically different.
4. To infer from the results of genetic analysis whether mating occurs at swarming sites in *M. nattereri.*

7.2. METHODS

7.2.1. Collection of samples

Tissue samples were collected from *M. nattereri* at Box and Byfield mines (Fig. 2.1) during swarming catches in 2001 and at two maternity roosts (Elm Farm and Forest Farm) in summer 2002 (Fig. 5.3). Elm Farm is 9.5 km WNW of Byfield and 17.2 km WSW of Box. Forest Farm is 26.6 km ESE of Byfield and 21.8 km SE of Box. Elm Farm and Forest Farm are separated by 36.2 km. Bats were caught for sampling using harp traps and mist nets at the swarming sites (as described in Chapter 2), by harp trap across the roost exit at Elm Farm (Plate 7.1) and by hand net at the exit at Forest Farm.

Tissue samples were taken by wing-biopsy. The area was wiped with a Medi-Swab (Smith and Nephew) and a wing punch was taken using a sterile 3mm biopsy punch (Stiefel Laboratories). A new punch was used for each bat. Each tissue sample was stored in a fresh Eppendorf tube in alcohol (100% ethanol). All bats were also fitted with a unique ring to avoid replication (Chapter 4).

Bats were sampled under license from English Nature and the Home Office.

7.2.2. DNA analysis

Freda Marshall carried out the microsatellite analysis at Aberdeen University in collaboration with Prof. Paul Racey. DNA was extracted from each wing biopsy and genotyped at nine autosomal microsatellite loci and one sex-linked locus (Paur3).

Loci used were:

MM1, MM5	characterised by Petri *et al.* (1997) for *Myotis myotis*
NN8, NN18	characterised by Mayer *et al.* (2000) and Petri *et al.* (1997) for *Nyctalus noctula*
Paur3, Paur5, Paur6	characterised by Burland *et al.* (1998) for *Plecotus auritus*
E24, F19, H29	characterised by Castella & Ruedi (2000) for *Myotis myotis*

7.2.3. Data analysis

Allele frequencies, values of allelic diversity (numbers of alleles per locus) and observed (Ho) and expected (He) heterozygosity were computed with the program GENEPOP (Raymond & Rousset, 1995). Exact tests for departure from Hardy-Weinberg equilibrium (HWE) were calculated using a Markov chain method (following Guo & Thompson, 1992) and global tests for HWE were estimated by Fisher's method across all loci for each population, both with GENEPOP. Fisher's exact tests for linkage disequilibrium between loci pairs were also carried out in GENEPOP.

To test for differentiation between samples tests of allelic and genotypic differentiation were undertaken in GENEPOP. F_{IS} and F_{ST} estimates were computed according to Weir and Cockerham (1984) with the program GENETIX (Belkhir *et* al., 1997). 1000 permutations were performed to test whether the resulting pairwise F_{ST} estimate was significantly different from 0 and therefore indicative of genetic differentiation between populations. *P*-values were adjusted by Finner's method, in the program PEPI (Abramson & Gahlinger, 1999), to correct for multiple significance tests. Isolation by distance among the samples was assessed following the method of Rousset (1997), by plotting pairwise values of F_{ST} /(1- F_{ST}) against the natural logarithm of the corresponding geographical distance, however sample size was insufficient to conduct a Mantel test for correlation between genetic and geographic distances.

Plate 7.1. *M. nattereri* roost at Elm Farm, Burnett.
Top: harp trap in place for catching. The bats exit in top right hand corner of barn door.
Bottom: interior of barn, roost is at the roof apex.

7.3. RESULTS

A total of 159 samples were taken from the four study sites. 114 *M. nattereri* were sampled during the course of five occasions at Box and three occasions at Byfield, totaling 83 males and 31 females. Twenty adult female and two juvenile male *M. nattereri* were sampled from the colony at Elm Farm over the course of two occasions and 23 adult female *M. nattereri* were sampled from the colony at Forest Farm on one occasion. During analysis some samples returned missing or uncertain results at some loci; individuals with data missing at more than one locus were removed before analysis. Analyses were conducted on males and females at each swarming site as separate populations and on females at each maternity colony, thus giving six sample populations in total. Allele frequencies for each locus in each sample are given in Appendix 5.

7.3.1. Allelic diversity and heterozygosity

All loci were polymorphic with between 4 and 15 alleles per locus (Table 7.1). Mean allelic diversity ranged from 4.4 ± 1.17 among Byfield females to 8.1 ± 3.75 among Box males (Table 7.2). Allelic diversity was significantly correlated with sample size (Pearson's correlation coefficient $r = 0.845$, d.f. = 9, $P < 0.02$). Allelic diversity was comparable between females at Box and at the maternity colonies. Observed and expected heterozygosity were not significantly correlated with one another ($r = -0.008$, d.f. = 9, $P > 0.05$). Heterozygosity was also not correlated with sample size ($r = 0.537$, d.f. = 9, $P > 0.20$).

7.3.2. Hardy-Weinberg equilibrium and F_{IS} analysis

Departures from Hardy-Weinberg equilibrium (HWE) were found in Box males, Byfield males and Byfield females (Table 7.1). In Box males, for the two departures that were significant, fewer heterozygotes were observed than expected and for Byfield males there were significantly fewer heterozygotes at two loci and more at one locus, although in both categories there were four and six cases respectively where more heterozygotes were observed than expected though the differences were not significant (Table 7.1). Among Byfield females there were more heterozygotes than expected at only one locus, but this difference was not significant. Departures from global HWE for each population across all loci were significant for Box males, Byfield males and Byfield females (Table 7.2). Mean heterozygosity was greater than expected overall for both groups of males indicating out-breeding, but for Byfield females observed heterozygosity was significantly lower than expected suggesting an excess of homozygotes, but it must be remembered that they had the smallest sample size, which may have affected the outcome.

F_{IS} estimates detected significant correlation of genes within Byfield females at six loci and for all loci combined (Table 7.3), consistent with the departure from global HWE towards excess homozygosity noted above. Unlike the Hardy-Weinberg results, correlations of genes were found at one locus in Box females, at two loci in Elm females and at one locus in Forest females. However there were also negative values of F_{IS} in both maternity colonies indicating that overall there was no trend for either excess homozygosity or heterozygosity and random mating can be assumed (as expected under HWE). No significant estimates of F_{IS} were found for Box males or Byfield males despite the significant Hardy-Weinberg exact tests, however overall F_{IS} was negative for both male groups indicative of a trend towards heterozygosity excess.

7.3.3. Linkage disequlibrium

Global exact tests for linkage disequilibrium between pairs of loci across all populations revealed one significant value. Tests for pairs of loci calculated per population gave nine significant values out of 216 comparisons (10.8 are expected by chance at 5% level) therefore these are assumed to have arisen by chance.

7.3.4. Allelic and genotypic differentiation between populations

Tests for allelic differentiation and genotypic differentiation between pairs of populations proved non-significant at the majority of loci (7/9) indicating that in general populations are genetically similar. However at locus MM5, there was significant allelic and genotypic differentiation between Box females and Elm females, Box males and Elm females, Box males and Byfield males and between Elm females and Forest females. At locus F19, there was significant allelic and genotypic differentiation between Box males and Forest females, and significant genotypic (but not allelic) differentiation between Box females and Forest females and between Elm females and Forest females. These results show that some differentiation exists between the two widely spaced maternity colonies (Elm and Forest) and between the maternity colonies and the swarming sites.

Pairwise comparisons of F_{ST} with P values adjusted for multiple comparisons found no significant deviations from 0 hence no population pair can be said to comprise genetically differentiated populations (Table 7.4). Figure 7.2 indicates a trend for greater F_{ST} at greater geographic distance however this cannot be tested for statistical significance.

Table 7.1. Sample sizes (*N*), values of allelic diversity (A), expected (He) and observed (Ho) heterozygosity and departures from Hardy-Weinberg expectations ($P < 0.05$) calculated by exact tests. Non-significant results are represented by NS.

	Box ♂ only	Box ♀ only	Byfield ♂ only	Byfield ♀ only	Elm ♀ only	Forest ♀ only
N	50	25	21	5	18	24
MM1						
A	15	12	9	5	9	11
He	0.821	0.778	0.799	0.756	0.651	0.799
Ho	0.780	0.720	0.810	0.800	0.778	0.792
HWE	0.013	NS	NS	NS	NS	NS
MM5						
A	7	7	9	5	6	8
He	0.657	0.689	0.778	0.800	0.683	0.752
Ho	0.740	0.680	0.762	0.800	0.722	0.708
HWE	NS	NS	NS	NS	NS	NS
NN8						
A	9	8	7	5	7	6
He	0.753	0.718	0.825	0.844	0.757	0.749
Ho	0.860	0.840	0.952	0.800	0.833	0.750
HWE	NS	0.017	NS	0.007	NS	NS
NN18						
A	4	4	4	4	3	4
He	0.614	0.584	0.623	0.711	0.624	0.604
Ho	0.540	0.560	0.667	0.600	0.611	0.458
HWE	NS	NS	NS	NS	NS	NS
Paur5						
A	6	6	5	5	5	5
He	0.708	0.710	0.749	0.866	0.722	0.735
Ho	0.700	0.680	0.714	0.800	0.556	0.833
HWE	0.09	NS	0.027	NS	NS	NS
Paur6						
A	12	11	11	6	11	10
He	0.834	0.867	0.808	0.778	0.881	0.878
Ho	0.860	0.760	0.810	0.60	0.778	0.875
HWE	NS	NS	NS	NS	NS	NS
E24						
A	12	12	11	5	11	14
He	0.899	0.887	0.827	0.844	0.875	0.903
Ho	0.840	0.840	0.905	0.600	0.944	0.875
HWE	NS	NS	NS	NS	NS	NS
F19						
A	6	5	5	3	5	5
He	0.589	0.512	0.600	0.378	0.568	0.559
Ho	0.580	0.480	0.571	0.200	0.500	0.542
HWE	NS	NS	0.040	NS	NS	NS
H29						
A	6	5	5	4	4	6
He	0.672	0.592	0.649	0.778	0.656	0.716
Ho	0.740	0.400	0.857	0.200	0.778	0.708
HWE	NS	NS	0.033	0.010	NS	NS

Table 7.2. Mean allelic diversity, mean expected heterozygosity (He), mean observed heterozygosity (Ho) (all ± SD) and global Hardy-Weinberg exact test results (HWE). Non-significant results are represented by NS.

	Box ♂ only	Box ♀ only	Byfield ♂ only	Byfield ♀ only	Elm ♀ only	Forest ♀ only
Mean A	8.56 ± 3.68	7.78 ± 3.15	7.33 ± 2.74	4.66 ± 0.87	6.78 ± 2.95	7.67 ± 3.35
Mean He	0.73 ± 0.11	0.70 ± 0.13	0.74 ± 0.09	0.75 ± 0.15	0.71 ± 0.11	0.74 ± 0.11
Mean Ho	0.74 ± 0.12	0.66 ± 0.15	0.78 ± 0.12	0.60 ± 0.25	0.72 ± 0.14	0.73 ± 0.14
Global HWE	0.0476	NS	0.0170	0.0154	NS	NS

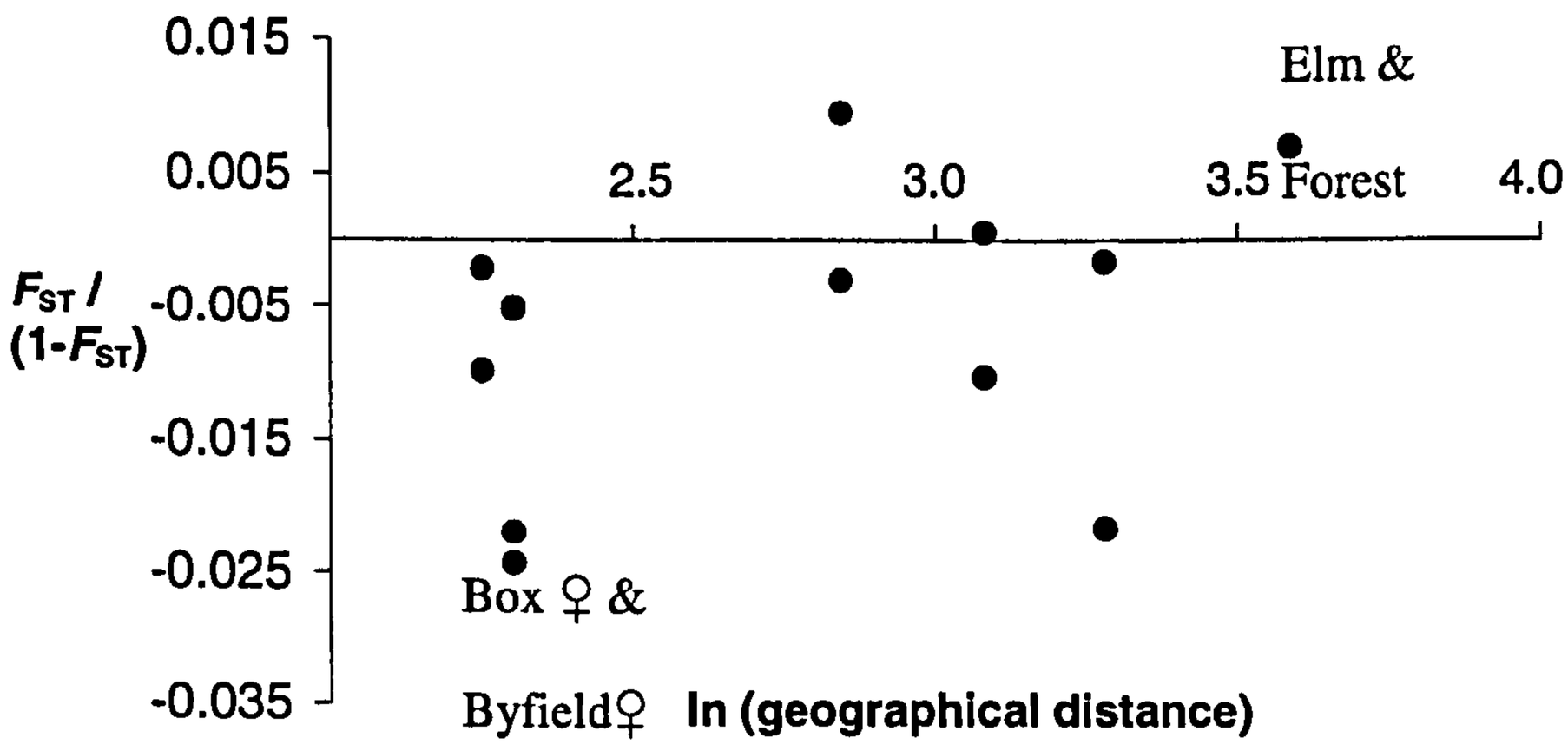
Table 7.3. F_{IS} estimates calculated separately for each locus and for all loci together. Significance levels are based on 1000 permutations. *$P < 0.05$, **$P < 0.001$.

Locus	Box ♂ only	Box ♀ only	Byfield ♂ only	Byfield ♀ only	Elm ♀ only	Forest ♀ only
MM1	0.051	0.076	-0.013	-0.067*	-0.202	0.009
MM5	-0.127	0.070	0.021	0.000	-0.060	0.059
NN8	-0.144	-0.175	-0.159	0.059	-0.104	-0.001
NN18	0.122	0.043	-0.073	0.172	0.021	0.245*
Paur5	0.012	0.043	0.048	0.086	0.236*	-0.137
Paur6	-0.031	0.126*	-0.001	0.250**	0.120*	0.003
E24	0.066	0.054	-0.097	0.314*	-0.082	0.032
F19	0.016	0.064	0.050	0.500**	0.123	0.031
H29	-0.102	0.193	-0.333	0.765**	-0.193	0.011
ALL	-0.014	0.055	-0.063	0.220*	-0.014	0.023

Table 7.4. Pairwise F_{ST} estimates calculated over 9 loci for swarming sites and maternity colonies. All were non-significant after adjusting P for multiple comparisons.

	Box ♂ only	**Box ♀ only**	**Byfield ♂ only**	**Byfield ♀ only**	**Elm ♀ only**
Box ♀ only	-0.003				
Byfield ♂ only	-0.005	-0.005			
Byfield ♀ only	-0.022	-0.025	-0.030		
Elm ♀ only	0.009	-0.003	-0.002	-0.016	
Forest ♀ only	0.0006	-0.010	-0.002	-0.023	0.007

Figure 7.2. Plot of pairwise F_{ST} / $1(F_{ST})$ against the natural logarithm of geographical distance between pairs of populations



7.4. DISCUSSION

7.4.1. Genetic diversity within populations

Allelic diversity and mean heterozygosity were generally high. Typically there are between five and ten microsatellite alleles per locus in large outbreeding populations and heterozygosity is often between 0.6 and 0.8 (Frankham *et al.*, 2002). Although mutation rates may vary between species making direct comparisons less valuable, in this study three loci had eleven or twelve different alleles in most samples. Box males gave 15 alleles at locus MM1 and Forest females gave 14 at locus E24. Allelic diversity was correlated with sample size. Ideally a larger sample would have been obtained for Byfield females before performing analyses but this was not possible. Therefore, results from Byfield females in particular should be interpreted with caution.

Departures from Hardy-Weinberg equilibrium and significant F_{IS} estimates were found, although the results were not consistent and appear to have been influenced particularly by the small sample size for Byfield females, in which the frequency of heterozygotes was reduced relative to Hardy-Weinberg equilibrium, indicative of non-random mating and inbreeding. Excess heterozygosity was observed in males at both swarming sites indicating inbreeding avoidance and out-breeding. This could potentially happen at swarming sites through mating with conspecifics from distant areas. Excess heterozygosity was not found for females at swarming sites or at the maternity colonies.

The result for Byfield females could indicate that females are from widely spaced maternity colonies some of which are differentiated from one another. A trend was found for isolation by distance and it is known that *M. nattereri* can travel greater distances than between the swarming sites and maternity colonies in this study hence there is potential for isolation by distance for even more widely spaced colonies still within the catchment of the swarming site. This could be explored by sampling females from more widely distributed colonies. Mitochondrial DNA haplotype diversity (as employed by Kerth *et al.*, 2000 for *M. bechsteinii*) could be used to better investigate the similarities and differences within and between maternity colonies and swarming sites and should be incorporated into further studies.

7.4.2. Genetic differentiation between populations

Tests for differentiation between populations showed no significant differences overall. Differentiation was found at three individual loci between the two maternity colonies and also between the maternity colonies and the swarming sites (particularly Box) and as mentioned above there was a slight trend toward more genetic differentiation between more

distant populations. Therefore, overall the populations are genetically homogeneous indicating that gene flow operates over a large area, consistent with mating at swarming sites within the catchment area. There was no evidence for differentiation between the swarming sites therefore it is assumed, because of their proximity, that bats are drawn from the same colonies within roughly similar catchment areas. However, from ringing and radio-tagging (Chapters 4 and 5) no movement was found between nearby sites.

The apparent lack of genetic differentiation could also be explained by random male dispersal from their natal areas to other regions before mating, maintaining a high level of gene flow. Most bats that have been studied, and indeed most mammals, show male dispersal and it is likely, though not inevitable that it also occurs in *M. nattereri.* Further studies in *M. nattereri* investigating natal philopatry and dispersal over a larger area incorporating many more maternity colonies would be beneficial. Sampling of juvenile males at nursery colonies would enhance information about males of the species, which is otherwise only gained through sampling at swarming sites when their origin is unknown.

7.4.3. Does *M. nattereri* mate at swarming sites?

The question of whether *M. nattereri* mates during swarming is essentially still unanswered. Mating groups in bat boxes have been suggested by Altringham & Bullock (1988) and Park *et al.* (1998). It is possible that there are two different mating strategies in operation. Perhaps those males that can defend mating roosts and attract a harem of females do so, and other males visit swarming sites to find females. Either way if males disperse from their natal regions, and accounting for the distances over which they have been shown to travel (Chapter 5) a high level of gene flow and low genetic divergence would be expected over the area considered in this study.

This study is somewhat limited by the small number of sub-populations and small sample sizes. A wider study covering a larger area encompassing more swarming sites and many more maternity colonies will be of greater value. Perhaps with a larger study differentiation may be seen between populations inhabiting the catchment areas of distant swarming sites.

CHAPTER EIGHT

GENERAL CONCLUSIONS

8. GENERAL CONCLUSIONS

This study has examined several aspects of autumnal swarming behaviour among bat species in Britain and has placed the findings in the context of their implications for the conservation of Britain's bats. Swarming was found to occur at underground sites, notably disused mines and tunnels, which are also used by bats for hibernation. However, catching during swarming invariably resulted in more species and more individuals than previously seen during hibernation counts. Thus, knowledge of the distribution and abundance of Britain's bat species can be enhanced by autumnal survey work at underground sites. To date hibernation counts have been the main method of monitoring underground bat sites and are a principal method of the National Bat Monitoring Programme (Walsh *et al.*, 2001). It is likely that the conservation value of many caves, mines and tunnels has been underestimated because some of the rarer species are presumed absent and numbers of hibernating vespertilionids actually counted are low because of their propensity to hide in crevices.

While swarming catches can obviously add much needed information about the use of a site by bats, they must be approached with caution. Initiating capture surveys at too many sites would be detrimental, and new or ongoing surveys must be closely controlled to avoid unnecessary disturbance to the bats. In addition, if useful data are to be gleaned from such surveys they must be properly coordinated and of a standard which ensures consistency of methods and data collection over time, so that results are comparable. Further exploration of the efficiency of different trapping methods would be of great benefit to further studies by allowing better extrapolation of the number of bats present but not caught. Ideally automatic loggers capable of distinguishing different species would be used at swarming sites to provide year-round information on the use of the site by bats so that catching would not be necessary. It is of vital importance that calibration of equipment remains identical over time and at different sites to enable comparisons. Data on environmental variables should be collected simultaneously to further elucidate why variation in activity is so great from night to night.

In the final report of the National Bat Monitoring Programme Walsh *et al.* (2001) discussed problems with monitoring maternity colonies of *M. nattereri* because of paucity of known roost sites. In this study I demonstrated how maternity colonies could be located for future study by radio-tagging bats from swarming sites. The techniques involved in tracking bats from swarming sites are necessarily expensive because of the distance over which bats travel, but can be effective. If it is essential that roosts are located, this would be a viable option, but, once again it is necessary to legitimise the potential disturbance before embarking on such studies.

The magnitude of swarming can be very great indeed, with up to a dozen species and hundreds of individuals present simultaneously at a site each night. Swarming in Britain, as elsewhere, is dominated by the genus *Myotis,* in particular *M. daubentonii* and *M. nattereri,* although substantial numbers of *M. bechsteinii,* one of Britain's rarest bats were also recorded. Year-round protection of swarming sites would certainly aid in the conservation of all swarming species. Male and female adults and juveniles were all caught suggesting that swarming involves all members of the population. The degree of bias in sex ratios differed between species, with the 'swarming' species (*Myotis* and *Plecotus* spp.) having highly male-biased sex ratios throughout the swarming season. In other species, most notably *Rhinolophus* spp. and *Pipistrellus* spp. sex ratios were at unity indicating that they are present for reasons other than mating and swarming, perhaps night-roosting or day roosting.

Nightly activity at swarming sites is negatively correlated with rainfall and positively correlated with maximum ambient temperature. Bats may only make the (often distant) journey to swarming sites on nights with good foraging and favourable weather conditions. Activity patterns throughout swarming suggest that few bats spend the day at swarming sites, and most arrive from the surrounding area within several hours of nightfall. Logged activity of bats was positively correlated with the number of bats caught confirming that loggers are a reliable alternative to catching when monitoring a swarming site. As mentioned above, further work is required to develop loggers capable of distinguishing between different species of bat.

The rate of return to swarming sites was in general low, particularly for females. Bats were more likely to return in subsequent years than within the same swarming season, although radio-tracking showed there to be considerable variation in return rate. Population sizes of *M. bechsteinii, M. daubentonii* and *M. nattereri* were estimated as approximately 150, 900 and 4000 respectively using mark-recapture techniques, but these estimates are crude and should be considered as a rough guide to the number of bats visiting the main study site. Better methods of population estimation for bats should be sought, although the number of recaptures is so low as to be limiting.

Minimum catchment area of the main study site was estimated to be 254 km^2 for *M. daubentonii* and 497 km^2 for *M. nattereri.* The maximum range recorded for each species was 35.1 and 36.1 km respectively; therefore the potential catchment area may exceed 4100 km^2. The catchments identified for both species were predominantly areas of mixed agriculture. Analyses identified a preference by *M. nattereri* to roost in areas with arable and pastural agriculture and to forage in broad-leaved woodland. Parkland, woodland and open water

habitats were most common around roosts of *M daubentonii.* Fidelity was seen to home ranges and roosts at this time of year, disputing the idea that bats wander randomly before hibernation. The journey to and from a swarming site is a deliberate move that must fulfill an important function in the bats' lives.

With the exception of one ringed individual found hibernating, no bat visited another swarming site during autumn, demonstrating a high degree of fidelity both during swarming and during hibernation. Hibernation counts at Savernake confirmed that bats swarming at a site later hibernate at the same site, and this is presumed also to be the case at the other sites although *Myotis* bats were seldom seen during hibernation. Such fidelity raises concern over the preservation of such sites, as bats may be inflexible should the site they know be destroyed. Lack of movement to other sites suggests that, if females are guiding their young to show them potential hibernacula, juveniles will have a limited choice of hibernacula.

Male bats are in peak reproductive condition when swarming during autumn, supporting the mating hypothesis for swarming. The order of sexual readiness corresponds with the order of swarming in the *Myotis* species. In general, those males in more advanced reproductive states were in better body condition than those with no reproductive development or less advanced development. Some juvenile males became mature in their first autumn.

Genetic diversity in *M. nattereri* was high and there was little differentiation between swarming site and maternity colonies, suggesting that gene flow operates over a large area, possibly consistent with large numbers of normally widely dispersed animals gathering at swarming sites for mating. However, this has not been conclusively proven and the same could arise from male dispersal over a large area. Further genetic studies should assist in understanding mating strategies in *M. nattereri* and other swarming species.

In summary, the function of swarming is still a matter of some debate and this study has contributed information to that debate. The presence of juveniles during swarming suggests that information transfer about hibernacula occurs, however the presence of sexually primed males in large numbers from a wide catchment area suggests that mating is also important. Preliminary genetic studies and knowledge of the size of the catchment area of swarming sites imply that gene flow is mediated at swarming sites, and consequently occurs over a very wide area. Maintenance of swarming sites for mating will no doubt aid in the preservation of genetic diversity among the bat populations served by those swarming sites.

Swarming may constitute an alternative mating strategy to those previously seen in bats whereby males gather and wait at a hotspot to copulate with visiting females. This system would work in species that cannot protect their investment in female mate choice or male defence of resources. Copulatory plugs do not form in most swarming species. However, several mating strategies may operate among such species, for example resource defence polygyny might be shown by those males that can defend roosts. If roosts or females are a finite resource, other males might follow an alternative swarming strategy by raping females at swarming sites. Some males might rape females during hibernation. In addition, it must be stated that the function of swarming may not be the same for all species. In some, prospecting for hibernation sites might take on greater importance and mating may have less significance than in other species.

The mating strategy might have evolved by males and females first prospecting for hibernation sites and subsequently males that mated while there gaining a reproductive advantage over males that did not. Males might be expected to visit on numerous occasions to obtain as many copulations as possible. To do this they would have to roost and forage close to the swarming site, in which case the population density would probably exceed the capacity of the habitat. Alternatively, they would have to make repeated long distance journeys that are energetically costly. Hence the number of times a male (or female) visits a swarming site during autumn is likely to be a trade-off between enhancing reproductive success and minimizing the costs of visiting the site. This is supported by the observation that a *M. daubentonii* living closer to the swarming site visited more often than one living far away. Further work on the mating systems of these species, in particular to confirm whether bats are mating at swarming sites by vaginal douching, would be beneficial to increasing understanding of this subject area.

In conclusion, autumnal swarming of bats has been a fascinating and difficult topic to study, presenting many challenges and each result has caused more questions to be posed. This study has contributed a great deal of information on bat behaviour and ecology, and on swarming in particular. Despite the mystery that still surrounds autumnal swarming it cannot be denied that it constitutes a major part of the life history of many temperate zone bat species.

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APPENDICES

1. Copy of paper based on Chapter 2.
2. Copy of paper based on Chapter 3 (proof).
3. Copy of paper based on Chapter 5 (proof).

4. List of all catch dates at the different swarming sites, hours spent trapping, number and type of traps used, total number of bats caught, bats per hour and bats per trap per hour.

5. List of (a) *M. daubentonii* and (b) *M. nattereri* fitted with radio-transmitters.

6. Minimum and maximum forearm lengths and masses recorded for *M. bechsteinii, M. brandtii, M. daubentonii, M. mystacinus, M. nattereri* and *P. auritus*.

7. Allele frequencies of ten microsatellite loci, calculated for samples from two swarming sites and two maternity colonies in southwest England.

SOME PARTS EXCLUDED UNDER INSTRUCTION FROM THE UNIVERSITY

Appended Papers!

Appendices 4

Appendix 4. List of all catch dates and locations, with hours spent trapping, number of traps used, total number of bats caught, bats per hour and bats per trap per hour. Location abbreviations as in Fig. 2.1. 1 and 2 are different entrances at the same site. MN = mist net, HT = harp trap.

Location	Date	N. hrs trapped	N. traps	N. bats caught	Bats / hr	Bats / trap / hr
Box 1	31.08.95	2.75	1 MN, 1 HT	43	15.64	7.82
Box 1	12.09.96	2.25	1 MN, 1 HT	47	20.89	10.44
Box 1	14.10.96	3.25	1 MN, 1 HT	41	12.62	6.31
Box 1	14.08.97	3.50	1 MN, 1 HT	32	9.14	4.57
Box 1	02.09.97	3.00	1 MN, 1 HT	25	8.33	4.17
Box 1	25.09.97	3.50	1 MN, 1 HT	61	17.43	8.71
Box 1	13.08.98	4.00	1 MN, 1 HT	38	9.50	4.75
Box 1	02.09.98	4.00	1 MN, 1 HT	22	5.50	2.75
Box 1	16.09.98	5.00	1 MN, 1 HT	84	16.80	8.40
Box 1	19.10.98	4.50	1 MN, 1 HT	12	2.67	1.33
Box 1	17.05.99	2.00	1 MN, 1 HT	3	1.50	0.75
Box 1	25.08.99	5.00	1 MN, 1 HT	51	10.20	5.10
Box 1	06.09.99	4.25	1 MN, 1 HT	136	32.00	16.00
Box 1	22.09.99	3.50	1 MN, 1 HT	19	5.43	2.71
Box 1	07.10.99	4.00	1 MN, 1 HT	21	5.25	2.63
Box 1	20.10.99	4.00	1 MN, 1 HT	14	3.50	1.75
Box 1	03.11.99	5.50	1 MN, 1 HT	58	10.55	5.27
Box 1	21.03.00	3.00	1 MN, 1 HT	29	9.67	4.83
Box 1	08.05.00	2.50	1 MN, 1 HT	1	0.40	0.20
Box 1	13.07.00	3.75	1 MN, 1 HT	1	0.27	0.13
Box 1	04.08.00	5.00	1 MN, 1 HT	21	4.20	2.10
Box 1	16.08.00	6.50	1 MN, 1 HT	112	17.23	8.62
Box 1	29.08.00	5.00	1 MN, 2 HT	148	29.60	9.87
Box 1	11.09.00	8.00	1 MN, 2 HT	147	18.38	6.13
Box 1	26.09.00	5.50	1 MN, 2 HT	43	7.82	2.61
Box 1	11.10.00	4.00	2 HT	8	2.00	1.00
Box 1	23.10.00	2.60	1 MN, 2 HT	8	3.08	1.03
Box 1	09.11.00	4.00	1 MN, 2 HT	21	5.25	1.75
Box 1	30.04.01	3.00	2 HT	21	7.00	3.50
Box 1	16.07.01	2.50	1 MN, 2 HT	3	1.20	0.40
Box 1	30.07.01	2.50	2 HT	46	18.40	9.20
Box 1	12.08.01	7.00	1 MN, 2 HT	61	8.71	2.90
Box 1	30.08.01	7.50	2 HT	87	11.60	5.80
Box 1	16.09.01	11.50	2 HT	207	18.00	9.00
Box 1	03.10.01	8.00	2 HT	81	10.13	5.06
Box 1	18.10.01	6.75	2 HT	76	11.26	5.63
Box 1	01.11.01	6.50	2 HT	12	1.85	0.92
Box 1	27.03.02	4.00	2 HT	36	9.00	4.50
Box 1	11.04.02	4.00	2 HT	32	8.00	4.00
Box 1	30.07.02	2.00	2 HT	9	4.50	2.25
Box 1	11.08.02	3.75	2 HT	17	4.53	2.27
Box 1	23.08.02	8.00	2 HT	159	19.88	9.94
Box 1	05.09.02	7.75	2 HT	88	11.35	5.68
Box 1	19.09.02	9.00	2 HT	215	23.89	11.94
Box 1	03.10.02	8.75	2 HT	149	17.03	8.51
Box 1	17.10.02	5.50	2 HT	33	6.00	3.00
Box 1	07.11.02	5.25	2 HT	16	3.05	1.52

Appendix 4. cont.

Location	Date	N. hrs trapped	N. traps	N. bats caught	Bats / hr	Bats / trap / hr
Box 2	22.09.98	4.50	1 MN, 1 HT	84	18.67	9.33
Box 2	02.09.99	4.75	1 MN, 1 HT	22	4.63	2.32
Box 2	30.09.99	4.00	1 MN, 1 HT	15	3.75	1.88
Box 2	29.08.00	5.50	1 MN, 1 HT	88	16.00	8.00
Box 2	19.09.02	6.50	1 MN, 1 HT	67	10.31	5.15
Byf	25.05.00	3.00	1 HT	5	1.67	1.67
Byf	30.05.00	4.00	1 HT, 1 MN	22	5.50	2.75
Byf	07.08.00	7.00	2 MN, 2 HT	91	13.00	3.25
Byf	21.08.00	7.50	2 MN, 2 HT	44	5.87	1.47
Byf	04.09.00	5.00	2 MN, 2 HT	39	7.80	1.95
Byf	21.09.00	4.50	2 MN, 2 HT	13	2.89	0.72
Byf	02.10.00	4.25	2 MN, 2 HT	51	12.00	3.00
Byf	16.10.00	4.00	2 MN, 2 HT	36	9.00	2.25
Byf	08.11.00	4.00	2 MN, 2 HT	18	4.50	1.13
Byf	01.08.01	3.00	1 HT	19	6.33	6.33
Byf	28.08.01	7.00	2 HT	129	18.43	9.21
Byf	17.09.01	6.00	2 HT	57	9.50	4.75
Byf	11.10.01	5.00	2 HT	44	8.80	4.40
Byf	25.10.01	4.00	2 HT	13	3.25	1.63
Chi	03.09.96	4.00	2 MN, 1 HT	24	6.00	2.00
Chi	21.08.97	3.50	1 MN	8	2.29	2.29
Chi	22.08.98	3.50	1 MN	18	5.14	5.14
Chi	25.08.98	3.00	2 MN, 1 HT	9	3.00	1.00
Chi	17.04.00	3.25	1 HT, 1 MN	5	1.54	0.77
Chi	03.05.00	3.00	1 HT, 1 MN	8	2.67	1.33
Chi	21.08.00	6.25	2 MN, 1 HT	65	10.40	3.47
Chi	12.09.00	5.00	2 MN, 1 HT	70	14.00	4.67
Chi	21.09.00	5.00	2 MN, 1 HT	107	21.40	7.13
Chi	19.08.01	5.50	2 MN, 1 HT	34	6.18	2.06
Chi	27.08.01	6.75	2 MN, 1 HT	72	10.67	3.56
Chi	27.09.01	7.00	2 MN, 1 HT	69	9.86	3.29
Chi	12.10.01	5.00	2 MN, 1 HT	13	2.60	0.87
Chi	02.09.02	5.00	2 MN, 1 HT	61	12.20	4.07
Coc	29.08.98	4.00	2 MN	61	15.25	7.63
Coc	04.09.98	4.50	2 MN	43	9.56	4.78
Coc	11.09.98	4.00	2 MN	37	9.25	4.63
Coc	25.09.98	2.50	2 MN	19	7.60	3.80
Coc	06.11.98	3.00	2 MN	8	2.67	1.33
Coc	01.04.99	2.50	2 MN	7	2.80	1.40
Coc	01.05.99	1.00	2 MN	4	4.00	2.00
Coc	15.05.99	1.50	2 MN	7	4.67	2.33
Coc	19.08.99	3.50	2 MN	20	5.71	2.86
Coc	15.10.99	3.50	2 MN	22	6.29	3.14
Coc	13.08.00	4.00	2 MN	8	2.00	1.00
Coc	09.09.00	4.00	2 MN	29	7.25	3.63
Coc	30.09.00	4.00	2 MN	28	7.00	3.50
Coc	28.07.01	4.00	2 MN	31	7.75	3.88
Coc	20.08.01	4.00	2 MN	19	4.75	2.38

Appendix 4. cont.

Location	Date	N. hrs trapped	N. traps	N. bats caught	Bats / hr	Bats / trap / hr
Dro	28.08.98	5.00	2 MN	38	7.60	3.80
Dro	24.09.98	1.00	2 MN	10	10.00	5.00
Far 1	17.08.00	4.50	2 MN, 1 HT	30	6.67	2.22
Far 1	29.08.01	4.75	2 HT	71	14.95	7.47
Far 1	28.03.02	2.50	2 HT	11	4.40	2.20
Far 1	13.08.02	2.75	2 HT	4	1.45	0.73
Far 1	12.09.02	6.25	2 HT	131	20.96	10.48
Far 1	23.10.02	4.00	2 HT	3	0.75	0.38
Far 2	05.09.00	3.00	1 MN, 1 HT	8	2.67	1.33
Far 2	16.04.02	3.25	2 HT	18	5.54	2.77
Fon	07.09.99	5.25	2 MN, 1 HT	48	9.14	3.05
Fon	23.09.99	3.00	2 MN, 1 HT	1	0.33	0.11
Fon	06.09.00	6.50	2 MN, 1 HT	87	13.38	4.46
Fon	03.09.01	3.00	2 MN, 1 HT	8	2.67	0.89
Fon	29.08.02	5.00	2 MN, 1 HT	36	7.20	2.40
Fon	12.09.02	6.50	2 MN, 1 HT	207	31.85	10.62
Fon	08.10.02	4.00	2 MN, 1 HT	53	13.25	4.42
Sav	14.09.99	2.00	1 MN	4	2.00	2.00
Sav	25.09.99		1 MN	7		0.00
Sav	15.10.99	7.00	1 MN	20	2.86	2.86
Sav	29.10.99		1 MN	0		0.00
Sav	17.03.00	3.00	1 MN	3	1.00	1.00
Sav	25.03.00	1.50	1 MN	3	2.00	2.00
Sav	30.03.00	2.50	1 MN	3	1.20	1.20
Sav	08.04.00	2.00	1 MN	4	2.00	2.00
Sav	20.04.00	1.00	1 MN	1	1.00	1.00
Sav	01.09.00	5.00	1 MN	14	2.80	2.80
Sav	08.09.00	9.50	1 MN	52	5.47	5.47
Sav	22.09.00	7.50	1 MN	66	8.80	8.80
Sav	14.10.00	4.00	1 MN	7	1.75	1.75
Sav	20.10.00	3.50	1 MN	5	1.43	1.43
Sav	27.10.00	8.50	1 MN	21	2.47	2.47
Sav	11.03.01	3.00	1 MN	8	2.67	2.67
Sav	17.03.01	3.00	1 MN	6	2.00	2.00
Sav	24.03.01	4.00	1 MN	10	2.50	2.50
Sav	30.03.01	2.00	1 MN	6	3.00	3.00
Sav	27.04.01		1 MN	0		0.00
Sav	24.08.01	6.50	1 MN	12	1.85	1.85
Sav	08.09.01	4.00	1 MN	6	1.50	1.50
Sav	21.09.01	7.50	1 MN	24	3.20	3.20
Sav	05.10.01	3.50	1 MN	4	1.14	1.14
Sav	13.10.01	8.50	1 MN	43	5.06	5.06
Sav	19.10.01	3.50	1 MN	4	1.14	1.14
Sav	02.11.01		1 MN	1		
Sav	15.03.02	3.00	1 MN	8	2.67	2.67
Sav	22.03.02	5.50	1 MN	20	3.64	3.64
Sav	28.03.02	2.00	1 MN	3	1.50	1.50

Appendix 4. cont.

Location	Date	N. hrs trapped	N. traps	N. bats caught	Bats / hr	Bats / trap / hr
Sav	23.08.02	8.00	1 MN	13	1.63	1.63
Sav	30.08.02	3.00	1 MN	4	1.33	1.33
Sav	06.09.02	6.50	1 MN	6	0.92	0.92
Sav	13.09.02	8.00	1 MN	36	4.50	4.50
Sav	20.09.02	3.00	1 MN	3	1.00	1.00
Sav	27.09.02	10.00	1 MN	71	7.10	7.10
Sav	04.10.02	9.00	1 MN	38	4.22	4.22
Sav	11.10.02	3.00	1 MN	5	1.67	1.67
Sav	18.10.02		1 MN	0		
Wes	30.08.95		1 HT	32		
Wes	17.08.96		1 HT	13		
Wes	20.08.96		1 HT	41		
Wes	28.08.96		1 HT	8		
Wes	31.08.96		1 HT	14		
Wes	09.09.96		1 HT	16		
Wes	17.09.96		1 HT	17		
Wes	09.08.97	4.00	1 HT	9	2.25	2.25
Wes	21.08.97	3.00	1 HT	6	2.00	2.00
Wes	30.08.97	4.50	1 HT	10	2.22	2.22
Wes	09.09.97	5.50	1 HT	38	6.91	6.91
Wes	26.09.97	6.00	1 HT	36	6.00	6.00
Wes	04.10.97	6.50	1 HT	33	5.08	5.08
Wes	17.10.97	6.25	1 HT	15	2.40	2.40
Wes	14.08.98	5.00	1 HT	22	4.40	4.40
Wes	07.09.97	4.00	1 HT	14	3.50	3.50
Wes	12.09.98	3.50	1 HT	11	3.14	3.14
Wes	15.10.98		1 HT	40		
Wes	05.09.99	4.00	1 HT	41	10.25	10.25
Wes	03.10.99		1 HT	15		
Wes	09.11.99		1 HT	12		
Wes	22.09.00	6.00	1 HT	76	12.67	12.67
Wes	23.10.00	5.00	1 HT	9	1.80	1.80
Wes	04.09.01	4.50	1 HT	13	2.89	2.89
Wes	06.10.01	5.00	1 HT	8	1.60	1.60
Wes	12.10.01	6.50	1 HT	33	5.08	5.08

Appendix 5a. Details of *M. daubentonii* fitted with transmitters during the study

Capture date	Sex	Age	Forearm (mm)	Mass (g)	Transmitter mass (g)	% of bat mass	Ring ID	RT ID	
29.08.00	M	A	36.2	7.4	0.48	6.49	U0335	D1	
29.08.00	M	A	36.3	7.8	0.48	6.15	U0342	D2	
29.08.00	M	A	38.3	9.3	0.48	5.16	U0353	D3	
29.08.00	F	A	37.6	7.8	0.48	6.15	U0338	D4	
29.08.00	F	A	34.8	8.7	0.48	5.52	U0369	D5	
11.09.00	M	A	36.9	7.6	0.48	6.32	U0573	D6	
11.09.00	F	A	38.2	12.2	0.48	3.93	U0592	D7	
12.08.01	M	A	37.6	8.1	0.52	6.42	U0296	**D8**	^
12.08.01	M	A	35.5	8.1	0.52	6.42	U0365	D9	^
12.08.01	M	A	37.4	7.9	0.52	6.58	U2566	D10	^
12.08.01	M	A	35.4	7.7	0.52	6.75	U2849	**D11**	
12.08.01	F	A	38.0	7.9	0.52	6.58	U2848	D12	
12.08.01	F	A	36.7	7.6	0.52	6.84	U2840	**D13**	
12.08.01	F	A	36.9	7.7	0.52	6.75	U2845	**D14**	
30.08.01	M	A	37.1	9.2	0.52	5.65	U3315	**D15**	
30.08.01	M	A	34.9	9.1	0.52	5.71	U0256	D16	^
30.08.01	M	A	37.1	8.8	0.52	5.91	U0359	**D17**	^
30.08.01	M	A	36.9	9.3	0.52	5.59	U3318	**D18**	
30.08.01	M	A	35.0	8.4	0.52	6.19	U0532	**D19**	^
30.08.01	F	J	38.4	8.7	0.52	5.98	U3320	**D20**	
30.08.01	F	A	37.4	8.0	0.52	6.50	U3343	D21	
30.08.01	F	A	36.8	8.2	0.52	6.34	U3325	**D22**	
16.09.01	M	A	37.2	8.2	0.52	6.34	U3388	**D23**	
16.09.01	F	A	38.6	10.5	0.52	4.95	U3392	D24	

KEY:

M = Male, F = Female

A = Adult, J = Juvenile

Ring ID = Number of bat ring.

RT ID = Number assigned for radio-tracking analysis. **Bold** = found after release

^ = bat caught and ringed on an occasion prior to that of transmitter attachment

Appendix 5b. Details of *M. nattereri* fitted with transmitters during the study

Capture date	Sex	Age	Forearm (mm)	Mass (g)	Transmitter mass (g)	% of bat mass	Ring ID	RT ID	
11.09.00	M	A	39.0	7.5	0.52	6.93	U0593	**N1**	
11.09.00	M	A	38.9	7.8	0.52	6.67	U2500	**N2**	
11.09.00	F	A	39.8	7.6	0.52	6.84	U0576	**N3**	
11.09.00	F	A	39.8	7.4	0.52	7.03	U0582	**N4**	
26.09.00	M	A	38.4	6.3	0.52	8.25	U2590	**N5**	*
26.09.00	M	J	38.5	7.1	0.52	7.32	U2550	**N6**	
26.09.00	M	A	39.7	7.9	0.52	6.58	U2592	**N7**	
26.09.00	F	A	38.5	8.7	0.52	5.98	U2601	**N8**	
11.10.00	M	A	38.6	8.1	0.52	6.42	U2668	**N9**	
11.10.00	M	A	38.8	7.6	0.52	6.84	U2568	**N10**	^
11.10.00	M	A	38.7	7.6	0.52	6.84	U2669	**N11**	
11.10.00	M	J	40.0	8.4	0.52	6.19	U2670	**N12**	~
11.10.00	F	A	40.1	9.2	0.52	5.65	U2672	**N13**	
16.09.01	M	A	39.8	8.8	0.52	5.91	U0370	**N14**	^
16.09.01	M	A	39.7	8.0	0.52	6.50	U0104	**N15**	^
16.09.01	M	A	40.0	8.0	0.52	6.50	U3391	**N16**	
16.09.01	F	A	40.1	8.3	0.52	6.27	U3274	**N17**	
16.09.01	F	A	40.6	9.8	0.52	5.31	U3403	**N18**	
16.09.01	F	A	40.2	8.5	0.52	6.12	U3258	**N19**	
03.10.01	M	A	38.9	7.5	0.52	6.93	T1032	**N20**	^
03.10.01	M	A	39.3	9.0	0.52	5.78	U4942	**N21**	
03.10.01	M	A	37.5	8.1	0.52	6.42	U0205	**N22**	^
03.10.01	M	A	40.1	8.1	0.52	6.42	U4929	**N23**	
03.10.01	M	J	40.5	7.2	0.52	7.22	U3439	**N24**	^
03.10.01	F	A	38.1	7.8	0.52	6.67	U4946	**N25**	
03.10.01	F	J	38.6	7.8	0.52	6.67	U4927	**N26**	
03.10.01	F	J	37.6	7.7	0.52	6.75	U4924	**N27**	
18.10.01	M	A	38.5	8.2	0.52	6.34	U4984	**N28**	
18.10.01	M	A	38.6	7.2	0.52	7.22	U2590	**N29**	^*
18.10.01	M	A	38.2	7.9	0.52	6.58	U0167	**N30**	^
18.10.01	M	A	39.5	9.4	0.52	5.53	U0190	**N31**	^
18.10.01	M	A	38.9	8.7	0.52	5.98	U4925	**N32**	^
18.10.01	F	A	40.7	10.2	0.52	5.10	U4991	**N33**	
18.10.01	F	A	40.4	8.1	0.52	6.42	U4988	**N34**	
18.10.01	F	A	39.6	9.4	0.52	5.53	U5007	**N35**	

KEY:

M = Male, F = Female,

A = Adult, J = Juvenile

Ring ID = Number of bat ring.

RT ID = Number assigned for radio-tracking analysis. **Bold** = heard after release

^ = bat caught and ringed on an occasion prior to that of transmitter attachment

* same individual

~ bat not found during tracking, caught by cat 27.11.01.

Appendix 6. Minimum and maximum forearm lengths (mm) and masses (g) for each species compared to ranges published in Greenaway & Hutson, 1990 (shown in brackets).

Species	Min. weight	Max. weight	Min. forearm	Max. forearm
Mbe	7.2 (7.0)	14.8 (13.0)	36.1 (38.0)	43.0 (47.0)
Mbr	4.1 (4.5)	9.2 (9.5)	32.1 (31.0)	36.9 (39.0)
Md	5.5 (6.0)	14.9 (12.0)	33.0 (33.0)	40.0 (40.5)
Mm	3.4 (4.0)	8.1 (8.0)	31.5 (30.0)	37.5 (37.0)
Mn	5.2 (6.5)	12.3 (12.0)	35.5 (36.0)	43.0 (43.0)
Pa	5.4 (4.0)	12.0 (12.0)	36.0 (34.0)	40.7 (42.0)

Appendix 7. Allele frequencies of ten microsatellite loci, calculated for samples from two swarming sites and two maternity colonies in south-west England

	Box ♂ only	Box ♀ only	Byf ♂ only	Byf ♀ only	Elm ♀ only	For ♀ only
Locus MM1						
(N)	100	50	42	10	36	48
154	0.010	0.000	0.000	0.100	0.000	0.000
158	0.050	0.060	0.024	0.000	0.056	0.042
160	0.360	0.440	0.381	0.500	0.583	0.417
162	0.010	0.020	0.000	0.000	0.000	0.042
164	0.010	0.020	0.000	0.000	0.000	0.000
166	0.110	0.060	0.143	0.100	0.028	0.104
168	0.020	0.100	0.000	0.000	0.056	0.042
170	0.050	0.020	0.000	0.000	0.056	0.062
172	0.010	0.000	0.024	0.000	0.000	0.000
174	0.020	0.020	0.048	0.000	0.000	0.062
176	0.160	0.140	0.190	0.200	0.111	0.125
178	0.100	0.060	0.095	0.000	0.028	0.062
180	0.050	0.020	0.071	0.100	0.028	0.021
182	0.030	0.040	0.024	0.000	0.056	0.021
184	0.010	0.000	0.000	0.000	0.000	0.000
Locus MM5						
(N)	100	50	42	10	36	48
135	0.000	0.000	0.095	0.000	0.000	0.042
136	0.000	0.000	0.000	0.000	0.000	0.021
137	0.000	0.000	0.048	0.000	0.028	0.000
138	0.010	0.000	0.024	0.000	0.000	0.000
139	0.000	0.000	0.000	0.100	0.000	0.000
141	0.030	0.060	0.095	0.000	0.250	0.042
143	0.000	0.020	0.024	0.000	0.000	0.000
145	0.330	0.320	0.167	0.300	0.139	0.271
147	0.480	0.460	0.429	0.400	0.500	0.396
149	0.070	0.080	0.071	0.100	0.056	0.167
151	0.060	0.040	0.048	0.000	0.000	0.042
155	0.020	0.020	0.000	0.100	0.000	0.021
157	0.000	0.000	0.000	0.000	0.028	0.000
Locus NN8						
(N)	100	50	42	10	36	48
152	0.000	0.020	0.000	0.000	0.028	0.000
154	0.090	0.080	0.095	0.200	0.056	0.125
156	0.060	0.060	0.095	0.000	0.056	0.042
158	0.150	0.080	0.143	0.100	0.111	0.083
160	0.150	0.260	0.214	0.300	0.361	0.125
162	0.440	0.460	0.310	0.300	0.333	0.438
164	0.060	0.020	0.119	0.000	0.056	0.188
166	0.020	0.020	0.024	0.100	0.000	0.000
168	0.020	0.000	0.000	0.000	0.000	0.000
170	0.000	0.000	0.000	0.000	0.000	0.000

Appendix 7. cont.

	Box ♂ only	Box ♀ only	Byf ♂ only	Byf ♀ only	Elm ♀ only	For ♀ only
Locus NN18						
(N)	100	50	40	10	36	48
111	0.160	0.160	0.175	0.300	0.194	0.208
113	0.550	0.600	0.525	0.500	0.528	0.583
115	0.250	0.200	0.225	0.100	0.278	0.146
117	0.040	0.040	0.075	0.100	0.000	0.062
Locus Paur3						
(N)	49	50	20	10	36	48
226	0.633	0.800	0.550	0.900	0.667	0.750
228	0.286	0.120	0.250	0.000	0.222	0.104
230	0.000	0.060	0.100	0.000	0.028	0.042
232	0.020	0.000	0.050	0.100	0.000	0.021
234	0.061	0.020	0.050	0.000	0.083	0.062
236	0.000	0.000	0.000	0.000	0.000	0.021
Locus Paur5						
(N)	96	48	42	10	36	48
222	0.010	0.021	0.000	0.000	0.000	0.000
224	0.354	0.396	0.405	0.200	0.444	0.396
226	0.208	0.292	0.190	0.200	0.222	0.292
228	0.094	0.104	0.143	0.200	0.111	0.125
230	0.302	0.146	0.214	0.300	0.194	0.146
232	0.031	0.042	0.048	0.100	0.028	0.042
Locus Paur6						
(N)	100	50	42	10	36	48
152	0.050	0.020	0.000	0.000	0.083	0.021
154	0.070	0.140	0.119	0.000	0.056	0.083
156	0.060	0.020	0.024	0.100	0.028	0.000
158	0.020	0.020	0.048	0.000	0.028	0.021
160	0.070	0.140	0.048	0.100	0.083	0.167
162	0.060	0.060	0.024	0.000	0.111	0.104
164	0.040	0.080	0.119	0.100	0.111	0.083
166	0.360	0.280	0.405	0.500	0.278	0.250
168	0.090	0.120	0.071	0.100	0.139	0.083
170	0.070	0.060	0.048	0.000	0.056	0.104
172	0.100	0.060	0.071	0.000	0.000	0.083
174	0.010	0.000	0.024	0.100	0.028	0.000

Appendix 7. cont.

	Box ♂ only	Box ♀ only	Byf ♂ only	Byf ♀ only	Elm ♀ only	For ♀ only
Locus E24						
(N)	100	50	40	10	36	48
202	0.010	0.000	0.000	0.000	0.000	0.000
210	0.000	0.000	0.000	0.000	0.000	0.042
214	0.000	0.020	0.000	0.000	0.000	0.021
216	0.070	0.080	0.025	0.000	0.056	0.062
218	0.140	0.100	0.225	0.300	0.083	0.062
220	0.150	0.200	0.100	0.200	0.167	0.229
222	0.130	0.180	0.250	0.100	0.278	0.083
224	0.140	0.140	0.125	0.300	0.111	0.167
226	0.080	0.100	0.075	0.000	0.028	0.062
228	0.040	0.100	0.050	0.000	0.083	0.062
230	0.070	0.020	0.050	0.000	0.111	0.021
232	0.100	0.020	0.050	0.100	0.028	0.062
234	0.010	0.020	0.025	0.000	0.000	0.021
236	0.060	0.020	0.025	0.000	0.028	0.062
238	0.000	0.000	0.000	0.000	0.028	0.042
Locus F19						
(N)	100	48	42	10	36	48
188	0.010	0.021	0.000	0.000	0.000	0.000
196	0.030	0.000	0.048	0.000	0.028	0.062
198	0.230	0.229	0.214	0.100	0.083	0.146
200	0.590	0.646	0.595	0.800	0.639	0.646
202	0.020	0.021	0.048	0.000	0.111	0.000
204	0.120	0.083	0.095	0.100	0.139	0.062
206	0.000	0.000	0.000	0.000	0.000	0.083
Locus H29						
(N)	96	46	38	10	36	48
167	0.052	0.043	0.079	0.100	0.083	0.062
169	0.260	0.326	0.289	0.300	0.167	0.292
171	0.000	0.000	0.026	0.000	0.000	0.021
173	0.417	0.500	0.421	0.400	0.528	0.438
175	0.010	0.022	0.000	0.000	0.000	0.083
177	0.250	0.109	0.184	0.200	0.222	0.104
179	0.010	0.000	0.000	0.000	0.000	0.000

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